Thompson Yates and Johnston Laboratories report

University of Liverpool


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THOMPSON YATES AND JOHNSTON
LABORATORIES REPORT

THE RT. HON. WALTER LONG M.P.

WILLIAM JOHNSTON

Frontispiece

THE
THOMPSON YATES AND JOHNSTON LABORATORIES REPORT

EDITED BY

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WITH

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WITH ILLUSTRATIONS AND PLATES

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PART I

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SINCE going to press we regret to learn of the death of our esteemed friend,

PROFESSOR NOCARD

An account of his life and career will appear in Part II of this volume.

CONTENTS

PREFACE

The munificent gift of Mr. WILLIAM JOHNSTON has placed beside the existing Thompson Yates Laboratories a laboratory for Bio-Chemistry, Tropical Medicine, Experimental Medicine, and Comparative Pathology. These laboratories are occupied and directed by workers who have already collaborated in the Thompson Yates Reports. It has, therefore, seemed as natural as it is desirable to now issue the work emanating from the two sets of laboratories in a single publication. The present volume is the first of a new series that we hope may continue to flow from the Thompson Yates and Johnston Laboratories in conjunction, evidencing the utility and fruitfulness of the generous gift with which the public-spirited founders have equipped the University of their city.

THE
OPENING OF THE JOHNSTON LABORATORIES

THESE new Laboratories devoted to Medical Research were formally opened on the 9th May by the Rt. Hon. WALTER LONG, President of the Local Government Board, in the presence of a distinguished gathering of medical representatives of British and foreign countries, and of a large number of the leading citizens of Liverpool. The following were amongst those who were present :—Professor NOCARD, Professor WEIGERT, Professor BLANCHARD, Professor PERRONCITO, Professor BOTTAZZI, Professor VON HANSEMANN, Professor UHLWORM, Drs. RAVENEL, ZIMMERMANN, WÜRTZ, BRUMPT, and POLAILON; Sir MICHAEL FOSTER, Sir DYCE DUCKWORTH, Professors CLIFFORD ALLBUTT, SCHÄFFER, GOTCH, SIDNEY MARTIN, BRADFORD, STIRLING, DELÉPINE, TREVELYAN, WALLER, THOMPSON, Drs. COPEMAN, BULSTRODE, MOTT, DAWSON WILLIAMS, MACMUNN, STEEGMANN, MANBY, Mr. F. J. WILLIS, Mr. WILLIAM JOHNSTON, Sir JOHN BRUNNER, Mr. E. K. MUSPRATT, Sir ALFRED JONES, Mr. JOHN HUGHES, Mr. SUTTON TIMMIS, Mr. JOHN RANKIN.

DESCRIPTION OF THE LABORATORIES

The entire block has taken eleven months to construct and to completely finish from the date of laying the foundation. The rapidity of construction has been of immense service in enabling students to take advantage without delay of the greatly increased accommodation, in attracting workers, and in enabling Professors ROSS and MOORE, the Director of the Cancer Research (the Lecturer in Expermental Medicine), Dr. GRÜNBAUM, and the Lecturer in Comparative Pathology, Dr. ANNETT, to start their respective lines of investigation.

The rapid progress was facilitated by the simplicity of the plan of the Laboratories. The essential points aimed at where—(1) maximum amount of light; (2) no dark corners, corridors, or waste spaces of any kind; (3) walls rendered impermeable by being covered by white-glazed tiles; (4) floors and working benches covered with a new material named lito-silo, which is impermeable and is moulded to sinks and fittings so that joints are abolished; (5) absence of internal partition walls, their places being taken by 7-feet screens of wood and glass, thus giving complete ventilation to each floor and contributing to the light; (6) simplicity of the gas, water, and electric light for each floor, so as to ensure immediate accessibility.

The laboratories were designed for post-graduate class work and for special research, the rooms or cubicles for the latter being very numerous on every floor.

Laboratory of Bio-Chemistry

The Bio-Chemical Laboratory occupies the entire top floor, and consists of four rooms fitted up solely for research work upon chemical problems connected with the various departments of biological science.

The principal room is 60 feet long and 30 feet wide, and is well lighted by ten large windows, 12 feet by 9 feet, the small remaining wall space being faced with white-glazed tiles, a wall cupboard being fixed to each pier to hold stock reagents and standard solutions. A complete belt of benches runs round the walls, and the middle of the room is practically divided into four working compartments by two large **H**-shaped benches, having an outside measurement of 22 feet by 16 feet. By this arrangement the laboratory is divided into bays in which investigators can work surrounded on all sides by working benches. The floor of the rooms and the tops of the benches are constructed of polished lito-silo, a material which lends itself well to such purposes on account of its resiliency, warmth, and non-absorbent properties. Drawers and presses are fitted underneath the benches for the storage of materials and apparatus, a free space being, however, left in the middle of each working space for convenience in sitting. Two large fume chambers, 9 feet by 3 feet, are built into the central benches, one at each end of the room, and contain six gas jets, regulated from outside, so that the fittings cannot be attacked by the fumes. Sinks are arranged in the wall benches opposite each pier, and each of the large central benches is provided with eight sinks. In addition, there are two large sinks placed one at either end of the room for washing glass apparatus. Steam is provided by a main pipe carried up the track of the lift and conducted along the wall on one side of the room for a distance of 18 feet, distribution taps with screw-down valves being provided at intervals of 3 feet for the attachment of steam baths and other heating appliances. The steam supply is also made use of for the production of distilled water by means of a suitable apparatus.

Opening from the south end of the large room is the Professor's Research Room, measuring 20 feet by 16 feet, provided with working bench, fume chamber, and cupboards.

To the east of this room, and communicating both with it and with the large room, is the Balance Room, provided with slate slabs for the support of the balances, let into the wall to ensure greater steadiness. The walls of this room are lined with bookcases, and there are writing tables under the windows.

The fourth room, situated on the west side of the Professor's room is designed as a working library for the use of workers in the laboratory, and is fitted up with bookcases and a writing table.

PLATE I

LABORATORY OF EXPERIMENTAL MEDICINE AND CANCER RESEARCH

LABORATORY OF COMPARATIVE PATHOLOGY

LABORATORY OF BIO-CHEMISTRY

LABORATORY OF TROPICAL MEDICINE

The whole laboratory is admirably lighted by large windows, and is fitted with the electric light. It is warmed by hot water, and ventilated by the upper parts of the windows and by extraction shafts arranged down the centre.

This is the first laboratory that has been constructed in Great Britain solely for carrying out research work in bio-chemistry.

Laboratories of Experimental Medicine and Cancer Research

The department of Experimental Medicine is housed on the first floor. One end of the rectangle is taken up by the Director of the Cancer Research. This room has window benches along the south and east sides, while in the centre are two tables, fitted with drawers, and with sinks at each end. The room has been equipped with all necessary apparatus for the histological and experimental investigation of cancer. Attention may be drawn especially to a very fine microscope by Swift, and a new mincing machine by Cogit, of Paris, by which tissues can be reduced to a state fine enough for injection with an ordinary syringe.

In the machine room in the basement is a centrifuge and disintegrator belonging to the equipment of the Research.

The other (north) end of the rectangle is divided into two private research rooms, each having two windows. The intervening portion is divided by a narrow corridor, on the east side of which are (1) the attendants' compartment, with large sink for washing up, shelves, etc., and a large slate on which are placed the autoclave and Koch's sterilizer connected with the laboratory steam supply ; (2) the incubator room, which is glazed up to the ceiling, in it are placed the incubator and the hot-air sterilizer, on slabs, and the glass-blower's table ; (3) a small room at present used for stores.

On the west side, next the entrance door and just opposite the Director's room, is the electrical room. In this is placed out of reach and danger an induction X-ray coil, giving a 24-inch spark ; while on the floor is a series of accumulators, charged from the main, for working the coil. A complete outfit for X-ray work is provided ; also a small resonator for applying the high frequency current and a large solenoid within which the patient can be placed. A new model arc lamp for investigating the therapeutic action of light, taking a current of fifty amperes, is also fixed in this room. It can be carefully darkened if necessary. Beyond this is a room for experimental pathology, and after this another research room. The Director's, the incubator, and the electrical room are glazed to the ceiling ; the others are separated by screens or cupboards, seven feet high, and the corridor is formed in the same way. There is consequently through ventilation for the whole floor. The fume chamber is placed in the corridor, and ventilates into the main flue.

Belonging to the Cancer Research is also a greenhouse, in which animals accustomed to a tropical climate can be kept.

Laboratories of Tropical Medicine

These are situated on the first floor of the Johnston Laboratories, and are contained within a large room, about 95 feet long and 35 feet broad. The main part of the room is devoted to students, but three chambers are partitioned off for the special use of persons who wish to do research work in connexion with Tropical Medicine and Parasitology, each of these rooms having all fittings and appliances for this purpose. One end of the floor contains the professor's room and the incubator room. The whole is walled with white glazed tiles. The floor is rendered impermeable by a layer of deep red lito-silo. Along the walls of the whole floor there runs a bench for microscope work, covered with a thick layer of sea-blue lito-silo. Electric light, gas, and water are suitably fitted at distances of every four yards of this bench for the use of students. The partitions of the room are fitted with cupboards, in which the museum of tropical diseases, which the school is now purchasing, is being arranged. Steam is also laid on to the laboratory, which possesses all the necessary apparatus. There is accommodation for quite forty workers in the new laboratory of the school.

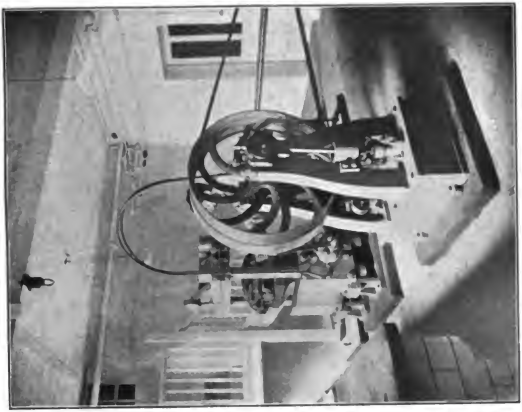
Laboratories of Comparative Pathology

The Institute of Comparative Pathology occupies the basement of the new Johnston Laboratory, and comprises the lecturer's private laboratory, a general laboratory, incubating room, sterilizing room, and a *post-mortem* room.

The laboratories are fitted with window benches and tables, and have a complete outfit of gas, water, and electrical fittings ; the walls are tiled with white glazed tiles, and the floors and bench tops are covered with lito-silo. Each side of the partitions dividing the area into sections is fitted with cupboards, which will be largely utilized for museum purposes. The *post-mortem* room will be fitted with a *post-mortem* table for large animals.

These laboratories will be furnished for lectures, demonstrations, and practical work in the subjects of comparative pathology and bacteriology, and also for the testing of various vaccines and sera prepared at the Institute's Farm Station. Facilities will also be afforded for acquiring the technique necessary for the manufacture of these products, and also for research work. Practical instruction in the physiological and pathological actions of these and allied substances will also be provided.

The farm in connection with the Institute is favourably situated in a most suitable and accessible agricultural district in North Cheshire. It has been provided with laboratories, fitted up with modern scientific appliances and apparatus for the production on a large scale of vaccines and sera. A general bacteriological laboratory, an incubating room, mixing, distributing, and sterilizing rooms, and a separate room specially set apart for the preparation of calf-lymph vaccine, and another room for plague prophylactic and serum, make up the laboratory accommodation.



200-TON PRESS IN THE MACHINE ROOM

MACHINE ROOM

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The preparation of the various sera and vaccines will be carried on at the farm station, and opportunities will be afforded to those interested in the subject to acquire the principles of their manufacture, and also to avail themselves of the practice in veterinary science at the out-patient department shortly to be established.

Machine Room

A large room has been fitted in the Thompson Yates laboratories with apparatus for the use of the various research departments. Shafting running the length of the room is driven by a 10 horse-power motor, and from the shaft is driven an hydraulic press giving a total pressure of 200 tons, a high-pressure filtering apparatus, and gas liquefier, and a powerful centrifuge. From a smaller motor and shafting is driven a large centrifuge, a vaccine mill, a bacterial mill and disintegrating apparatus. The shafting is so constructed that new apparatus can be added as occasion requires.

Manufacture of Media

A special room has been set apart for the preparation of media, which is now required in large quantities. A method which has proved very useful is the use of various coloured cotton plugs to distinguish the numerous sugar and other media.

OPENING SPEECHES

On the evening of the 9th May, a banquet, presided over by Mr. WILLIAM JOHNSTON, was given to Mr. WALTER LONG and the numerous distinguished guests who had come to Liverpool for the opening of the new Laboratories. The Lord Mayor of Liverpool, at the request of the Chairman, proposed the toast of the Right Hon. WALTER LONG; Sir MICHAEL FOSTER replied to the toast of Science and Commerce given by Sir ALFRED JONES; and Dr. RAVENEL, Professors NOCARD, WEIGERT, and PERRONCITO replied to the toast of 'Our Foreign Guests' given by Sir JOHN BRUNNER. The following speeches were delivered by Dr. RAVENEL and Professors NOCARD, WEIGERT, and PERRONCITO:—

DR. RAVENEL'S REPLY TO THE TOAST

MR. CHAIRMAN, MY LORD MAYOR, MR. LONG, AND GENTLEMEN

In the name of my country I beg to thank Sir JOHN BRUNNER most heartily for the warm terms in which he has spoken of the foreign guests, and especially for the most flattering references made by him to America, and beg to assure him that whatever feeling of being strangers we may have had on our arrival, was quite dispelled by the warm welcome accorded to us in his city as well as by his words to-night. I could well wish that the task of representing America had been entrusted to more able hands; I trust that I am not one of those guilty of the crime, often charged against my countrymen, of squeezing the eagle on every occasion to make him scream his loudest. Well, I confess to such faith in my country that I feel that the best we have, there is none too good to represent her on any occasion whatsoever. You can understand then what my feelings are in being asked to represent her on an occasion so memorable as this. Graced by an assembly of men, prominent in all walks of life, gathered together in celebration of the opening of laboratories, which I may, without exaggeration, say give the promise of the opening of a new era in Preventive Medicine. I have been asked to give some account of what is done in the United States of America for the cure of diseases of animals in general, and especially of those diseases transmissible to man. We have in Washington a central department, under the charge of the Secretary of Agriculture, known as the Bureau of Animal Industry, now under the able care of Dr. DANIEL E. SALMON, a veterinarian of distinction. In this department there are the following sub-divisions:—a Laboratory of Bio-Chemistry, under the charge of Dr. DE SCHWEINITZ, which employs, as a rule, from twelve to fifteen men; a Pathological division, under the charge of Dr. MOHLER, with about

PLATE III

PROFESSOR NOCARD

DR. RAVENEL

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the same number of workers as the dairy division, which has charge of matters pertaining to the dairy industry. In addition to these departments there are employed, under Dr. DE SCHWEINITZ, about 2,500 meat inspectors. Many employed in the examination of meat at the slaughter-houses, while others are employed in the microscopic division for the detection of trichinae, and other such diseases; many of the latter being women.

Besides this central bureau, each State has its own laws and regulations to meet local and individual necessities. For instance, in the State of Pennsylvania we have a State Live Stock Sanitary Board, consisting of the Governor of the State, a Secretary of Agriculture, a Dairy and Food Commissioner, and the State Veterinarian, and under this Board is a Bacteriological Laboratory, of which I have charge; in which diagnostic and research work is carried out. There are also in the State of Pennsylvania a Forestry Commissioner, an Entomologist, a Geologist, and a number of Chemists, so that it will be seen that the number of trained scientific men employed in our country is quite large. There is also in Pennsylvania a State College, in which especial attention is given to the testing of foods and fertilizers, and to the instruction of young men in the principles of scientific Agriculture and Dairy Farming.

The invitation to attend the opening of the Johnston Laboratories was especially a welcome one to me, and came like a command. Our recent responsibilities in the acquisition of the Phillipine Islands, in Cuba and Porto Rico, and the Isthmian Canal, as well as the closer relationships which have been established between my country and tropical countries in matters of trade, have forced upon us, so to speak, the necessity of careful study of the diseases incident to these climates, so that I immediately realized the great opportunity presented to me of learning from those who have for some years prosecuted studies so rich in results, as you have associated with you in Liverpool in your Tropical School. Your school has more than justified its existence, and where it not that it has such sober truth, it would seem fulsome for me to add to what has already been said in praise of the magnificent munificence of Mr. JOHNSTON in establishing the Johnston Laboratories. We can understand, however, the absolute confidence with which he has given this money when we glance over the history of your school for the past four years, and which in that short time has established for itself a reputation equalled by none in the world. In going through the laboratories as we have done to-day, I am sure that all of us foreigners, as well as your own people, must have received inspiration, and it was difficult to know what to admire most, the far-sighted generosity which had established these laboratories or the brain which had planned and equipped the building. We feel sure, however, that like the apparatus so substantial, solid, and of the best quality, the work turned out from these laboratories will be of an enduring and lasting character—a boon not to the University, not to Liverpool, not to England even, but to the whole world. The interest of other people in these laboratories is attested by the number of scientific

men who have come from great distances to attend your ceremonies. Their presence is an indication of that finest of all rivalries in which each nation strives to out-run the others in discoveries for the prevention of disease—discoveries which once made are not patented, nor copyrighted, nor controlled in any manner whatever, but given freely to suffering humanity of every nation and of every clime. The meeting this afternoon, as well as this gathering to-night, is unique as far as my experience goes in one other point, viz. :—The number of business men who are here with your scientific men, apparently all as deeply interested and concerned as are the scientific men themselves. It is an inspiring sight, and one full of hope for the scientific man of the future.

In conclusion, Mr. Chairman, I beg to thank you personally for myself, as well as for my country, for the generous hospitality which you have accorded me.

Professor NOCARD'S REPLY TO THE TOAST

Messieurs

Je m' excuse de parler en français ; j'ai pour cela une bonne raison ; c'est que je ne sais pas parler anglais ; je ne l'ai jamais autant regretté qu' aujourd' hui.

Pourtant, si je vous inflige l'ennui d'écouter un mauvais discours français, il ne faut pas trop m'en vouloir. C'est la faute de mon ami Boyce. Je l'avais prié de confier ce toast à mon collégue Blanchard ; Boyce n'a pas voulu ; il prétend que c'est le droit et le devoir du plus ancien.

Le droit d'ancienneté ! Comme on y renoncerait avec joie !

Pourtant Blanchard était bien mieux qualifié que moi pour parler au nom des Français ici présents.

D'abord, il a le don des langues ; il parle l'anglais, l'allemand, l'italien, presqu' aussi bien que le français.

En second lieu, c'est à coup sûr, parmi nous tous, celui qui connait le mieux l'oeuvre considérable que vous avez accomplie ; c'est lui qui vous l'a fait connaître en France et qui nous a donné le désir de la voir de plus près.

Enfin c'est surtout à lui que nous devons d'avoir pu jeter en France les bases d'une Ecole de Médecine Coloniale analogue, de loin—oh ! de bien loin hélas !—à celle de Liverpool.

A tous ces titres, Blanchard vous aurait dit, beaucoup mieux que moi, combien nous avons été touchés de votre gracieuse invitation, combien nous sommes heureux d'avoir vu ces superbes laboratoires dont vous a dotés la munificence éclairée de vos concitoyens, combien nous vous sommes reconnaissants du grand example que vous donnez au monde et des idées fécondes que nous emporterons de Liverpool.

J'étais chargé tout spécialement de vous apporter les compliments et les voeux de l'Institut Pasteur de Paris et de l'Ecole Vétérinaire d'Alfort. MM. Duclaux,

PLATE IV

PROFESSOR BOTTAZZI

PROFESSOR WEIGERT

To face p. 9

Roux, Laveran, Metchnikoff cette pléiade de savants dont nous sommes si fiers en France, et en qui revit l'esprit de Pasteur, m'avaient chargé de vous exprîmer tous leurs regrets de ne pouvoir assister à votre belle fête.

Mais puisqu' aussi bien, mon ami Boyce m'en a fait une obligation, c'est au nom de tous mes compatriotes ici présents—MM. Blanchard, Würtz, Brumpt, et Polaillon, qui appartiennent tous à l'Ecole française de Médecine Coloniale—que je vous prie d'agréer l'expression bien sincère et bien cordiale, de nos remercîments de nos félicitations et de nos voeux.

Messieurs, L'Ecole de Médecine Tropicale de Liverpool a déjà rendu d'éminents services à la science et à l'humanité ; en attribuant le prix Nobel de Médecine au plus illustre d'entre vous, le monde savant l'a reconnu d'une façon éclatante. Aujourd' hui que l'Ecole de Liverpool dispose de ces admirables moyens de travail que font les 'Thompson Yates et les Johnston Laboratories,' nul doute qu'il n'en surgisse bientôt de nouvelles découvertes, aussi belles que le premières, aussi glorieuses pour la science, aussi fécondes pour les progrès de la civilisation.

Messieurs, Je bois a la prospérité de l'Ecole de Médecine Tropicale de Liverpool et je vous demande la permission de résumer mon toast en la vieille devise de nos Universités latines.

Vivat, Florat et Crescat!

Professor WEIGERT'S REPLY TO THE TOAST

Hochverehrte Festgenossen!

Vor etwa siebzig Jahren, zu der Zeit also, als Schwann seine epochemachenden Arbeiten über die Zellenlehre veröffentlichte, da waren für unsere Wissenschaft noch recht glückliche Zeiten. Man brauchte nur, wie Meister Henle sich einmal ausdrückte, mit dem Fingernagel über irgend etwas hinwegzukratzen und das Abgekratzte unter das Mikroscop zu bringen, sogleich hatte man eine Entdeckung gemacht. Man führte eben damals den Krieg gegen die Feinde der Menschheit, gegen die Krankheiten (und um einen solchen directen oder indirecten Krieg handelte es sich ja immer), so zu sagen mit der Hand und den einfachsten Werkzeugen, gerade wie in alter Zeit die Kriege der Menschen untereinander nur mit der Faust und den einfachsten Waffen ausgefochten wurden. Die Zeiten haben sich seitdem gründlich geändert. Was man mit jenen einfachen Mitteln entdecken konnte, ist längst entdeckt, aber es ist immer noch ungeheuer viel zu entdecken übrig, und wenn man jetzt 'die Natur ihres Schleiers berauben' will, so muss man es eben doch 'mit Hebeln und mit Schrauben' zu erzwingen suchen (Faust), man muss allem möglichen Mittel der Mechanik, der Physik und der Chemie verwenden, um Entdeckungen auf dem Wege der Beobachtung oder des Experiments zu Stande zu bringen. Während die alten Forscher für ihre so oft

ja bahnbrechenden Arbeiten nur eines kleinen Zimmers und einiger einfacher Apparate bedurften, so sind jetzt zur Forschung und nun gar für den Unterricht mächtige Institute nöthig, ausgestattet mit reichlichen kostbaren Einrichtungen und Apparaten, gerade wie die eigentlichen Kriege ja auch zur Vorbereitung und zur Führung der raffinirtesten technischen Apparate und der ausgeklügeltesten Mittel bedürfen. Welch ungeheure Summen diese menschen*vertilgenden* Kriege dadurch verschlingen, das weiss ja ein jeder, aber auch die menschen*erhaltenden*, die wir zuführen haben, bedürfen unter den gegenwärtigen Verhältnissen grosser Mittel, wenn diese auch im Vergleich zu jenen gar sehr bescheiden genannt werden können. Es ist nun für die Menschheit im Allgemeinen höchst erfreulich und für ein specielles Gemeinwesen ausserordentlich ehrenvoll, wenn sich in einem solchen Männer finden, die in hochherziger und verständnissvoller Weise grosse Summen spenden, damit gut ausgestattete Forschungs und Lehrinstitute gegründet werden können. Zur Einweihung eines solchen neuen Institutes, das der Munificenz eines Liverpooler Bürgers seine Entstehung verdankt, sind wir aus weiter Ferne eingeladen worden, und wir sind der Einladung gern gefolgt. Es ist mir nun ein besonderes Bedürfniss vor Ihnen, geehrte Festgenossen, auch im Namen *meiner* Landsleute unseren herzlichsten Dank auszusprechen, einmal für die liebenswürdige Einladung überhaupt, sodann für die so überaus freundliche Aufnahme, die wir hier gefunden haben, und für die schönen Worte, mit denen uns Sir John Brunner begrüsst hat. Diesem speciellen Danke möchte ich aber noch einen allgemeineren hinzufügen, nämlich den für das gute Beispiel, das die Liverpooler Bürger allen andern Bürgern gegeben haben, und von dem nur zu wünschen ist, dass es aller Orten recht eifrige Nachahmung finden möge.

I drink to the health of the Maecenas of Liverpool, and especially the health of Mr. Johnston.

Professor PERRONCITO'S REPLY TO THE TOAST

Illustri Signori, e Chiarissimi Colleghi!

Sono orgoglioso di portarvi il saluto della mia patria, che ebbe pochi giorni addietro la fortuna di ospitare il vostro Augusto Sovrano Re Edoardo VII.

Noi Italiani serbiamo la piu grande venerazione pel popolo Inglese, lo ammiriamo spesso nelle sue potenti iniziative pel progresso delle Scienze, delle arti, delle industrie del commercio e dell' agricoltura. Con quanta soddisfazione io abbia quindi accolto l'invito di partecipare a questa grande solennità dovuta allo spirito intraprendente degli Inglesi, e alla munificenza del benemerito Signor Johnston, potete immaginarvelo pensando all' amore che si deve portare alle officine della scienza e del lavoro.

Sua Eccellenza il nostro Ministro della Pubblica Istruzione Nunzio Nasi,

PLATE V

To face p. 10

penetrato della grande importanza che avrà questa nuova Scuola nel mondo scientifico, e del bene che ne potrà ridondare all' umanità intiera, mi ha affidato l'alto onore di rappresentare il suo Ministero, e l' Università Italiana a questa festa. Quindi Ministero ed Università Italiane oggi festeggiano con voi il grande avvenimento che formerà esempio non morituro di amore alla scienza ed al progresso mondiale.

La Scuola di Medicina Tropicale, largamente dotata ed organizzata nel porto che tiene il primo posto pel commercio e le comunicazioni con tutto l'orbe terraqueo, costituirà il faro massimo del progresso scientifico ed umanitario.

L'Università di Liverpool dalle nuove forze e dalla potente Istituzione, acquisterà quell' alta posizione che le sapranno creare la sapienza dei docenti, la proverbiale munificenza Inglese, ed un governo forte e libero sotto la dinastia di un Re ed Imperatore dottissimo.

Non vi è chi non scorga nell' istituzione del Laboratorio di Medicina Tropicale una iniziativa della piu alta importanza, massime ora che i rapporti commerciali, ed i mezzi di trasporto sono così facili tra le popolazioni nordiche e quelle del sud dei due emisferi ; fatto che se da un lato è fonte di benessere economico, dall' altro puo essere causa di piu facile diffusione dei morbi infettivi. Collo studio delle speci parasitarie che trovano ambiente piu adatto nelle zone calde dove l'umidità e la temperatura sono elementi importantissimi per la loro coltura fuori dell' organismo dell'uomo, e delle speci animali utili, si oppone un' argine potente al loro sviluppo e alla loro diffusione.

La Bio-Chimica che scruta la composizione degli elementi anatomici in rapporto alle loro proprietà vitali, e studia i prodotti degli organismi grandi e piccoli benefici e viventi parasitari, ne valuta l'importanza per stabilire norme curative e preventive generali e sicure contro i morbi epidemici ed epizootici.

E da essa la scienza si attende nuovi progressi che possiamo prevedere importantissimi.

La Patologia sperimentale che studia le malattie producendole artificialmente per indagarne meglio la essenza l'andamento e gli esiti per una cura eventualmente specifica, in questi laboratorii potrà piu rapidamente progredire a benefizio dell' umanità e della ricchezza mondiale.

La Patologia comparata studiando con opportuni mezzi le malattie delle diverse speci animali, e nelle piante affretterà il giorno in cui noi saremo in pieno possesso del '*nosce te ipsum*' per combattere i mali nella loro origine, redimere le regioni ora malefiche, e transformare il tutto a beneficio dell' umanità progredita.

Signori ! Interprete dei sentimenti del Ministro che ho l'onore di rappresentare e di tutti gli Italiani porto anch' io un brindisi quale tributo di riconoscenza al Signor Johnston per la sua opera di alto interesse internazionale coll' augurio che questi laboratorii dischiudano e rischiarino presto nuovi orizonti pel benessere universale.

Finisco con un Evviva al Popolo Inglese ed al suo grazioso Sovrano !

THE NATIONAL IMPORTANCE OF THE STUDY OF TROPICAL VETERINARY MEDICINE

THE NATIONAL IMPORTANCE OF THE STUDY OF TROPICAL VETERINARY MEDICINE

Being a speech delivered by Professor Nocard *at the Conference of Tropical Medicine held in Liverpool, May* 11, 1903

Ce qui m'a le plus frappé pendant mon séjour à Liverpool, c'est l'alliance intime qui s'y'est établié entre le Commerce et la Science ; alliance féconde dont bénéficient largement la civilisation et l'humanité tout entière.

Chez nous, en France, loin d'encourager les hygiénistes, les commerçants les considèrent comme des géneurs, sinon comme des ennemis et l'on voit, de temps à autres, les agriculteurs recevoir à coups de fourche ceux qui sont chargés d'appliquer la loi sanitaire qui n'a pourtant pas d'autre but que de sauvegarder les intérêts de l'agriculture—Vous, Messieurs les commerçants de Liverpool, vous avez compris que la marchandise qui coûte le plus cher, c'est encore la vie humaine et que les savants et les hygiénistes sont les plus précieux de vos auxiliaires.

Honneur à vous ! Puisse votre example être suive de l'autre côté de la Manche !

On vous a dit sur tous les tous depuis 3 jours les beautés de l'oeuvre que vous avez accomplie ; voulez-vous me permettre de vous signaler une lacune qu'il vous sera d'ailleurs facile de combler ?

Votre école de médicine tropicale est tres bien outillée pour doter vos colonies d'excellents médecines ; mais il semble que vous n'ayez pas songé à y préparer de bons Vétérinaires coloniaux. Et cependant la chose en vaut la peine !

Pour tous les pays neufs—et ce sont les plus fructueux pour les marchands hardis et entreprenants que vous êtes—les voies de communication sont plus que rudimentaires ; le roulage y est inconnu ; les seuls moyens de transport sont les bêtes de somme ; à défaut de bêtes de somme, il ne reste que l'homme et vous savez mieux que moi le prix exhorbitant du transports à dos d'homme.

Votre Livingstone a dit quelque part que la 'tsé-tsé' était le plus grand obstacle a la pénétration de la civilisation dans l'afrique centrale ! C'est que toutes les bêtes de somme sout sensibles au 'Nagana' que leur inoculent les piqûres de la 'Tsé-tsé' ; et c'est aussi que la 'Tsé-tsé' a fait échouer lamentablement tant d'expéditions, tant d'explorations admirablement préparées

L'étude Scientifique des maladies des animaux est donc tout aussi nécessaire que celle des maladies de l'homme pour aider aux progrès de la civilisation qui sont lies si étroitement à la prospérité du Commerce.

Permettez moi de vous citer un fait récent, qui interesse tout particulièrement l'Angleterre, et qui vous montrera d'une façon saisissante l'exactitude se cette proposition.

En temps normal, c'est à Madagascar que les agriculteurs de l'Ile MAURICE s'approvisionnent du Bétail nécessaire au travail de la terre ou au transport des récultes. Pendant la guerre Sud-Africaine, l'Angleterre a utilisé pour la nourriture de son armée presque tout le Bétail disponible à Madagascar.

Les Mauritiens ne pouvant lutter contre la concurrence que leur faisait la métropole aut dû se pourvoir ailleurs. Beaucoup ont eu l'ideé de faire venir des boeufs Indies qu'ils pouvaient acheter à meilleur compte. En Septembre, 1901, un navire chargé de boeufs indies dût être umiss en quarantaine à son arrivée à Fort-Louis, pour cette raison qu'un certain nombre de boeufs étaient morts pendant la traversée ; quelques unes étaient encore malades ; les Vétérinaires chargés du service sanitaire les examinèrent à diverses reprises, firent l'autopsie de plusieurs, sans réussir à déterminer la nature et la cause de la maladie ; puis, après un certain temps, la mortalité ayant cessé, les malades paraissant guéris, on leva *l'embargo* et l'on rendit a la libre désposition des importateurs le reste de la cargaison.

Or qu' est-il arrivé ?

Partout où les hasards de la vente avaient disséminé les survivants, on vit bientôt leurs compagnons d' écurie tomber malades, et succomber avec des symptômes et des lésions identiques à ceux observés pendant la traversée ou pendant la quarantaine. Bien plus, la maladie ne se borna pas à frapper les boeufs ; elle frappa également et d'une façon beaucoup plus grave les chevaux et les mulets, puis se propagea rapidement, dans toutes les directions, sur toute la surface de l'Ile, en sorte que les agriculteurs se demandaient avec anxiété comment ils allaient faire la récolte de cannes a Sucre.

Cette maladie si grave, ou en a longtemps méconnu la nature ; ce n'est que vers la mois d'avril, 1902 qu'un médecin, le docteur LESUR, examinant au microscope le sang d'animaux malades appartenaut a l'un de ses amis, y constata la présence de nombreux parasites filiformes, extrcmement mobiles, qu'il put aisément assimiler au Trypanosóme du *Nagana* de l'Afrique australe ou du *Surra* de l'Inde.

Monsieur LAVERAN et moi avons confirmé ce diagnostic, après étude de nombreuses lames de sang qu'ou nous envoya de MAURICE.

Vous savez que le *Nagana* est propagé par la 'Tsé tsé' qui, puisant dans la peau des animaux malades du sang chargé de parasites, l'inocule par ses piqûres dans la peau des animaux sains.

La 'tsé-tsé' n'existe pas dans l'Inde Anglaise ; c'est un autre insecte suceur, le 'tabanus tropicus,' qui parait jouer le même rôle qu'elle. 'A MAURICE, on ne connait ni la tsé-tsé ni le taon des tropiques ; mais il y existe un grand nombre de mouches piquantes qui tourmentent les animaux au travail et, parmi elles, il en est une, le 'stomoxys nigra,' qui parait bien ctre l'agent principal de la propagation de la maladie.

Jusqu à présent ou ne connait ancun moyen de traiter efficacement le *Nagana* ou le *Surra* ; le seul moyen sûr d'éviter lés ravages de ces maladies, consiste donc à empêcher l'importation d'aminaux malades. Si les autorités de MAURICE eûssent fait abattre à l'arrivée tous les survivants de la cargaison infectée, l'île eût échappé à l'effroyable épizootie qui menace de la ruine ses industries agricoles.

Malheureusement, les agents du service sanitaire avaient une instruction technique insuffisante ; ils n'avaient jamais vu le *Surra ;* ils ignoraient par quels moyens simples on peut le reconnaître, même chez des aminaux qui ne présentent aucun symptome apparent et, quand la maladie a été reconnue, il était trop tard pour enrayer son extension ; déjà l'île entière était infecteé.

Eh bien, supposez que les mauriciens aient pris l'habitude d'envoyer leurs vétérinaires, tous les 2 ou 3 ans, faire un stage de quelques semaines dans une école comme la vôtre, pour se retremper aux sources, pour se mettre au courant des progrès de la science, nul doute qu'ils n'aient réussi à dépister l'existence du *Surra* sur les boeufs suspects et à faire l'économie de l'épizootie actuelle qui constitue un véritable désastre !

Par cet exemple, vous jugerez sans doute, Messieurs de Liverpool, que votre Ecole de Médecine tropicale qui a déjà rendu tant de services ne doit pas seulement vous préparer de bons médecins coloniaux, mais qu'elle doit être aussi largement ouverte aux Vétérinaires qui se destinent au service des Colonies.

Soyez sûrs que l'excèdent de dépenses qui en résultera pour votre budget, vous sera remboursé au centuple.

ON THE SYNTHESIS OF FATS ACCOMPANYING ABSORPTION FROM THE INTESTINE, AND ON THE LIMITATIONS OF SYNTHESIS BY ENZYMES AND BY LIVING CELLS, RESPECTIVELY

ON THE SYNTHESIS OF FATS ACCOMPANYING ABSORPTION FROM THE INTESTINE, AND ON THE LIMITATIONS OF SYNTHESIS BY ENZYMES AND BY LIVING CELLS, RESPECTIVELY

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IT is now generally accepted that the neutral fats of the food are completely split up in the course of intestinal digestion, by the lipase of the pancreative juice, into free fatty acids and glycerine, and that the fatty acids are then rendered soluble by the bile salts and lecithin, either in the form of free acids or as alkaline soaps. So that all the constituents formed in fat digestion are finally taken up by the columnar cells in soluble form.[1]

It has long been known that the main part, if not all, of the absorbed fat leaves the intestinal wall by the channel of the lacteals, and that the fatty chyle contained in these vessels, after traversing the abdominal lymphatic glands, is poured by the thoracic duct into the systemic circulation. Now somewhere along this course a synthesis occurs of the fatty constituents back again into neutral fats, for MUNK[2] has shown that when fatty acid, instead of neutral fat, is administered by the mouth, a synthesis has been effected by the time the absorbed fatty acid has entered the thoracic duct, whereby from 90 to 95 per cent. of the free acid has been combined to form neutral fat. It is also worth noting, in view of the discussion which will be presented later on in this paper of the character of the synthesis here brought about, that it is unnecessary to feed glycerine along with the fatty acid ; that is to say, somewhere between intestine and thoracic duct, glycerine is either carried in or synthetically formed from other constituents, and united to the fatty acid to form neutral fat. This experiment, therefore, casts doubt on the view that the fatty acid in the normal course of digestion of fat is entirely re-united to the same glycerine from which it was detached in the intestine. For it is probable that the rates of absorption of glycerine, and fatty acid or soap, respectively, from the intestine are not quite identical ; and although it is possible that these constituents after absorption are stored by cells somewhere along the channel of absorption, and re-united to each

1. Moore & Rockwood, *Proc. Roy. Soc.*, vol. lx, 1897, p. 438 ; *Journal of Physiology*, vol. xxi, 1897, p. 58 ; Moore & Parker, *Proc. Roy. Soc.*, vol. lxviii, 1901, p. 64 ; Pflüger, *Arch. f. d. ges. Physiologie*, Bd. lxxxii, 1900, S. 303, 381 ; Bd. lxxxv, 1901, S. 1 ; Bd. lxxxviii, 1902, p. 299, 431 ; Bd. xc, 1902, S. 1.

2. *Virchow's Arch.*, lxxx, 1880, S. 17 ; xcv, 1884, S. 452.

other, it is also possible that the glycerine absorbed may either be used as a source of energy by these cells, or allowed to pass on, and the fatty acid later united in the cell to other glycerine synthetically formed by chemical processes going on in the cells in which the synthesis of fat from fatty acid or soap occurs.

Although it is fairly certain that a hydrolysis of fat occurs in the intestine and a re-synthesis before the thoracic duct is reached, there is, at the present time, no clear experimental evidence as to the situation in the course of absorption at which the synthesis is effected, or as to the agency by which the synthesis is brought about, that is to say, as to whether it is due to an intra-cellular enzyme, or is a process in which the living protoplasm acts as the energy transformer.

The experiments recorded in this communication were designed with the object of shedding light on these points.

There are two chief places at which the lymph, carrying the absorbed fatty constituents, either comes into intimate relationship with cells, or is forced to pass through them, viz., first, in the intestinal wall itself, in the columnar lining cells and adenoid tissue of the villus lying between these and the central lacteal, and secondly, in the abdominal lymphatic glands, through which the chyle all passes on its way to the thoracic duct. It is hence at these two situations that chemical changes may be expected to occur, and the suggestions naturally present themselves of examining the composition of the chyle as regards fatty constituents, before and after passing these points, and of studying the action of these tissues upon the constituents involved in the synthesis of neutral fats.

There is no question that the intestine during fat absorption contains both fatty acids and soaps in solution, and Munk has shown, as above stated, that the lymph of the thoracic duct during fat absorption contains nearly all its fatty matter as neutral fat; but the most difficult point in the course, from which to obtain lymph in sufficient quantity for chemical analysis of its fatty constituents, is the portion lying between the intestine and abdominal lymphatic glands, for the mesenteric lacteals are far too small to admit a cannula.

As far as the writer is aware, no analyses have hitherto been published of the fatty matter contained in the chyle of the mesenteric lacteals, but, by a method, which will be described later, a sufficient supply of fatty chyle was obtained to demonstrate most clearly that already, in the mesenteric lacteals, practically all the fatty matter is present as neutral fat.

It has long been known, from histological evidence, that a change occurs in the absorbed fatty constituents in the columnar cell. For such cells, taken some hours after a fatty meal, when examined under the microscope after appropriate fixing and staining, are seen to be filled, except in the striated border, with globules of varying size, which present all the histological appearances and reactions of fatty material.

In the first place, however, such histological preparations do not conclusively prove that *neutral fat* is synthesized in the epithelial cell, since the appearances might be reproduced by insoluble fatty acids deposited in the cells. In the second place, even assuming the appearances to be due to neutral fat, they give no quantitative information as to whether any fraction of the fatty constituents, absorbed in soluble form from the intestine, passes through the columnar cell unmodified into the lacteals to be changed in the abdominal lymphatic glands or elsewhere. Thirdly, as has been supposed by some writers on the subject, such a deposit in the intestinal cells as has been observed microscopically might be only a temporary one, the fat being again hydrolyzed to pass out towards the lacteal, so that a constant stream of fatty acid and glycerine has been assumed to diffuse out from the attached border of the cell. Nor, assuming as proven that the fatty constituents enter the columnar cell in soluble form, does the presence of a white milky fluid in the lacteals, containing fatty globules when viewed under the microscope, demonstrate that neutral fat has been synthesized in the intestinal cell. In the first place, as has been remarked above, regarding the appearances in the columnar cell, all such effects might be reproduced by free fatty acid in suspension, and, in the second place, no quantitative information is given by such observations as to the amount of conversion from soluble into insoluble constituents.

Turning next to the nature and place of synthesis, as shown by the action of cells and extracts of cells concerned in fat absorption upon the constituents of neutral fats, we find on reviewing the literature that a good deal of attention has been given to this aspect of the subject by previous workers.

C. A. Ewald[1] was the first observer who claimed to have detected such a synthetic action *in vitro*, from products of the intestinal cell. In a preliminary paper, published in 1883, he states that he was stimulated by the account of Brown and Herron's work upon the inverting action of dried mucous membrane upon cane sugar, to test whether a similar dried preparation of the mucous membrane was capable of synthesizing fat from an aqueous solution of soap and glycerine at the proper temperature. This experiment with the dried cells failed, and the reason ascribed by Ewald, was that one has here to do with a synthetic operation, such as is peculiar to the metabolism of the living cell, and not a ferment action which would remain after the destruction of cell activity, as in the case of inversion of cane sugar.

Ewald accordingly turned his attention to the fresh mucosa, and in two experiments, in which he divided the intestinal mucous membrane in each case into four portions, one of which was treated alone as a contral, while to the other three portions soap and glycerine were added in appropriate quantities, he believed that he had observed in all but one case a considerable formation of neutral fat from fatty acid and glycerine.

1. *Arch. f. Physiol. u Anat., Physiol. Abth.*, Suppt. Bd. 1883, S. 302

Much more recently, HAMBURGER,[1] working chiefly with the large intestine, has brought forward evidence from a large number of experiments, claiming to show that synthesis of neutral fats can be brought about *in vitro*, by the separated intestinal mucosa.

Neither EWALD nor HAMBURGER worked with extracts free from cells, and their methods of working save in experimental details were the same, so that both sets of results can be conveniently discussed together.[2]

The method was essentially that of digesting a mixture of soap and glycerine in solution, in presence of intestinal mucosa finely minced up, for a variable period, obtaining an ethereal extract, and determining the amount of fatty acid in this by titration against a standard alkali.

The difference between the weight of the total ethereal extract and the weight of free fatty acid was taken to be neutral fat, and the difference between this figure, in the case of portions to which soap and glycerine had been added, and controls in which no soap and glycerine were added, was taken to represent neutral fat which had been formed during the experiment.

No direct determinations were made by saponification with alkali of the amount of neutral fat formed. Now, had such a course been taken in the experiments detailed later in this paper, as a perusal of the figures will show, a fallacious conclusion that fat was synthesized would in some cases have been the result. But the direct determinations show that this is not the case, and hence that probably in EWALD and HAMBURGER's experiments, also, the supposed fat synthesized was in reality soap dissolved by the ether used as an extractive.

In any case, the present experiments show no appreciable synthesis of neutral fat from soap and glycerine, either by the fresh intestinal mucosa or by cell-free extracts of it.

Other workers have turned from the direct synthesis of neutral fats and attempted instead to draw analogies from the synthesis of simpler esters.

Thus, KASTLE and LOEVENHART[3] digested a mixture of dilute butyric acid and ethyl alcohol with a fresh aqueous extract of pancreas, and were able to detect ethyl butyrate by its odour, and on the larger scale were able to obtain a few drops of a light oil with the odour and general properties of that ester. The reaction did not occur when boiled pancreas was used, and since, on the other hand, lipase can also be employed to convert ethyl-butyrate into butyric acid and ethyl alcohol, it becomes evident that the action is a reversible one.

1. *Arch. f. Physiol.*, 1900, S. 433.

2. The only essential difference was, that Hamburger evaporated to dryness before extracting with ether, while Ewald first filtered after digestion and then evaporated the filtrate to a small volume, and extracted with ether, adding this to an ethereal extract of the residue from filtration.

3. *American Chemical Journal*, 1900, Vol. xxiv, p. 491.

In a later paper, LOEVENHART[1] found a similar action in varying degree with extracts of intestinal mucosa, lymphatic glands, lymph, liver, kidney, submaxillary gland, lung, brain, adrenal and subcutaneous connective tissue. Similarly, HANRIOT[2] found that pancreatic lipase synthesized monobutyrin from butyric acid and glycerine.

It is, however, exceedingly dangerous, as is obvious on comparing the above-mentioned results with those obtained in the present investigation, to draw conclusions as to the hydrolysis or synthesis of one ester, from that of another.[3] This is true, even when all the constituents of the reaction are soluble, as even there the position of the point of equilibrium, as experiment shows, may vary enormously with different esters and be placed practically at either end point of the reaction ; but the proceeding becomes entirely misleading when the comparison is made between such dissimilar esters as the body fats and ethyl butyrate, where in the former action two of the three components are practically insoluble, and in the latter all three are easily soluble. Nor does it seem at all justifiable without experimental evidence to assume that the same catalyser, lipase, can in the intestine cause an equilibrium lying (in the case of the neutral fats) practically at the end point of complete conversion into fatty acid and glycerine, and within the cell cause a complete reversal into the neutral fat.

It is certainly true that the concentrations in the solution of the reacting units of the chemical system vary the position of the equilibrium point ; but it seems highly improbable that any such *complete* shifting as is required by the above theory of hydrolysis in the intestine and re-synthesis in the columnar cell by the same enzyme can be attained by changes in concentration alone.

Such a reversal is, however, possible in the living cell, where there is a supply of external energy, and means for its transformation.

It may be pointed out that such conditions can only very faultily be carried out *in vitro*, and hence it is not surprising that syntheses, such as are undoubtedly carried out by the cell *in situ*, cannot be obtained with the detached cells in test tubes.

An attempt was made in certain of the experiments recorded below to supply such a source of energy by adding dextrose to the solutions, but even then no formation of neutral fat could be brought about.

The only action upon the soaps employed, which could be demonstrated with certainty, was a considerable conversion into free fatty acid. This result was invariably obtained, and is also observable in the experiments of EWALD and HAMBURGER, although little attention is bestowed upon it by these authors.

The action is, however, of some physiological importance ; in the first place, because it is probably the preliminary stage in that synthesis of fat which is

1. *American Journal of Physiology*, 1902, Vol. vi, p. 331.
2. *Comptes rendus de la Société de biologie*, 1901, p. 70.
3. This view is also held by LEWKOWITSCH, *Journ. of Soc. of Chemical Industry*, Vol. xxii, No. 2, 1903.

undoubtedly effected in the intestinal cells (*vide infra*). In the second place, the reaction has an importance as an indication of how the organism is protected from the entry into the systemic circulation of absorbed soaps, which, as was first demonstrated by MUNK,[1] act as virulent poisons when injected into the circulation, although perfectly innocuous when administered by the mouth.

This protective action of the cells, or of some product contained by them, is similar in function to the action of the intestinal cells upon albumoses.

This action upon soaps has been obtained with extracts of intestinal mucosa, abdominal lymphatic glands, and pancreas, and is most powerfully exhibited by pancreatic extracts. When a water-clear solution is prepared by dissolving two per cent. of sodium oleate in an extract of 1 in 10 of fresh pancreas, and the solution kept at body temperature, within a few minutes the fluid becomes turbid, and when examined under the microscope is seen to be full of minute highly refractile globules. On standing for some hours, a thick oily layer appears on the top, which, when separated by ether and titrated with decinormal alkali, is found to correspond with oleic acid. The separation is not due to acidity, for the pancreatic extracts employed were alkaline to phenol-phthalëin and remained so. The alkali separated from the soap does not become free in the solution, for the alkalinity of this does not increase during the reaction, but it appears to become stably combined with some substance present in the extract. This is further shown by the fact that the extracts containing free fatty acid in suspension, or as a creamy or oily layer on the surface, can be evaporated down to dryness without recombination occurring, as must be the case if free alkali were present.

In the earlier experiments, a fear of such a recombination occurring led to the adoption of the method of extracting the fatty products, after a certain period of digestion, directly with ether, without previously evaporating to dryness.

It was found, however, that although the first extractions with ether by this method gave higher yields of fatty acid than the later ones, yet no definite end result could be obtained, since the extractions even after the sixth still yielded appreciable amounts of fatty acid.

This result is obviously due to the considerable solubility of ether in water, leading to an increase in the hydrolysis of the sodium oleate solution, and hence increasing the amount of oleic acid, which dissolved in the ethereal fraction.[2]

Accordingly, this method was subsequently abandoned, and the method of evaporating to dryness and then extracting with dry ether substituted, as was previously done by HAMBURGER.

The experiments by the first method have, however, a distinct qualitative value as a confirmation of the results obtained by the second method, for, although

1. *Arch. f. Anat. u. Physiol., Physiol. Abth. Suppl.*, 1890, S. 116.

2. This was shown by the control experiment that ether extracts small quantities of oleic acid when shaken up with solutions of sodium oleate in distilled water.

they do not show accurately the amount of free fatty acid formed, they very conclusively prove *that no neutral fat is formed.* For, while a considerable amount of soap is dissolved out from the dried extracts in the second method, which, if the attempt be made to obtain the amount of neutral fat, as in EWALD and HAMBURGER's calculations, by merely subtracting the amount of free fatty acid from the total ethereal extract, gives rise to the fallacious result that a considerable synthesis of neutral fat has taken place; in using the first method, namely, that of extracting without previous evaporation to dryness, practically no soap is dissolved by the ether, and the subsequent titration gives almost theoretical values for oleic acid, showing that the entire ethereal extract consists of fatty acid, and that no neutral fat is present.

If any neutral fat whatever had been formed by the previous digestion with the extracts of intestinal mucosa and other tissues employed, it would undoubtedly have been present in the ethereal extract, because olein is practically insoluble in water, but soluble in all proportions in ether, and hence would have been dissolved out even more readily than oleic acid, which has a slight solubility in water.

HAMBURGER, although he did not proceed to the direct determination of the neutral fat in the extracts he obtained, was aware of the solubility of soaps in ether, and sought to equalize this by adding, after evaporation, soaps to his controls. Even with this precaution, he found a greater weight of ethereal extract, after deducting free fatty acid, in those portions to which the soap had been added before boiling, and ascribed this excess to neutral fat.

A repetition of this procedure in the present series of experiments, did not give, however, a like result, the difference in weight was very slight, and in one case was in favour of the portion to which the soap had been added before digestion, and in the other, in favour of that to which the soap had been added after digestion.[1]

HAMBURGER's own results, moreover, are not uniform, for while a greater weight was found in some experiments, no appreciable change was found in others.

HAMBURGER supposes that the negative result is due to re-conversion into fatty acid during prolonged digestion of the neutral fat first formed.

Supported by the results of the method of direct estimation of the neutral fat, as well as by the results obtained by duplicating HAMBURGER's own method, we are of the opinion that the positive results of HAMBURGER are due to dissolved soap, which varied in amount in the different experiments.[2]

The hydrolysis of sodium oleate and accompanying liberation ot oleic acid, above alluded to, by extracts of pancreas, lymphatic glands, and intestinal mucosa, is remarkable in that it is not completely stopped by previous boiling of the extracts. In most cases the extent of hydrolysis is lessened by boiling, as will be seen by

1. See *Experiments* 1 and 2, Series ii.
2. It may be mentioned that Hamburger used *sapo medicatus*, while Sodium Oleate was used for the experiments described in this paper.

reference to the protocolls given below, and the extent of such diminution of action is variable, but even after boiling the extracts for five minutes, a considerable effect has in many cases been obtained, and that in cases where bacterial action has been carefully excluded. This is particularly observable in the extracts of intestinal mucosa and lymphatic glands, where the difference between boiled and unboiled extracts is less than in the case of the pancreas.

In fact, a considerable action has been observed in some cases after complete evaporation to dryness of the extract on a steam bath, on again adding water and soap and digesting at body temperature.[1]

It appears probable from this that the hydrolysis is not due entirely to an enzyme, but to some chemical substance with acid properties contained in the extracts which, at body temperature, slowly hydrolyses the soap and combines with the liberated alkali.

If, instead of *first* boiling the extract and then adding the soap after it has cooled to 36° C. and digesting at that temperature, the soap be added to the unboiled extract and then the temperature be raised as rapidly as possible to 100° C., a very rapid formation of free fatty acid occurs, and the fatty acid floats at once in an oily layer on the surface of the extract even before the boiling point is reached.

The extracts employed were in all cases alkaline to litmus, and the slight acidity to phenol-phthalëin which they showed, like nearly all animal extracts, was found by titration to be much less than equivalent to the fatty acid liberated.[2]

Excess of sodium carbonate stops the reaction, however, which does not occur when 0·5 per cent. of sodium carbonate is present in solution.

Experiments on the Nature of the Fatty Constituents Present in the Mesenteric Lymphatics During Fat Absorption

In these experiments the lymph was obtained from the lacteals at a period of five to seven hours after feeding on neutral olive oil.

The animals (dogs) were anaesthetized by a mixture of chloroform and ether, about five hours after feeding with the oil; the abdomen was then opened and the intestine drawn out in small loops consecutively, so exposing the lacteals, which stand out clearly like silk threads, from being filled with the white, milk-like chyle. This is the appearance presented by the lacteals when first seen, but in a very short time, not exceeding five to ten minutes, they become discharged, and fat absorption appears to stop almost entirely. In making the collections of lymph, now to be described, it is hence necessary to proceed quickly, for the absorption is not restored very rapidly on replacing the loop of intestine and temporarily closing the abdominal wound, and if

1. See *Experiment* 1, Series iii, Addendum 2.

2. In some of the experiments this acidity to phenol-phthalëin was slightly over neutralized by addition of sodium carbonate solution.

time be spent in this procedure, the whole of the lacteals may soon become emptied. It is also necessary to disturb and expose as little of the intestine as is possible at any one time, and then when the flow of lymph from one loop has been exhausted to replace that and pull out a fresh loop.

The flow of lymph may be much augmented by gently kneading the portion of the intestine from which the lacteal arises.

Even employing all these precautions, the interval after opening the intestine during which the lymph can be collected is a short one, amounting to about fifteen minutes.

The vessels are too small for the insertion of a cannula, and hence the lymph was collected by free incision of the vessel, taking care not to injure the accompanying small blood vessels, and allowing the lymph to collect upon the mesentery. This it does in large drops, which are collected by small glass tubes made capillary at both ends by drawing out a glass tube in the blow-pipe flame. The lymph runs readily into these tubes when they are held horizontally, with one of the capillary ends in the drop of lymph. In this way from one to two dozen small tubes can be charged with lymph. Another method which was employed for collection was to suck the lymph up by means of a glass tube drawn out fine at one end, and then blow out into a porcelain capsule.

The subsequent process of analysis consists in weighing the tubes and then placing them in a test tube containing ether. In a short time the contents of the small tubes become transparent from the solution of the fatty constituents, and soon after the lymph flows out and the watery portion collects at the bottom, while the fatty constituents are dissolved by the ether. The tubes are taken out and washed twice with ether, the washings being added to the first portion of ether used for extraction. The tubes are then dried and weighed, the difference between this weight and the previous one then obviously gives the weight of lymph collected in the tubes.

The amount which can be thus collected is small, but fortunately determinations of fatty acid and fat can be made so closely, and the results lie so preponderatingly on the side of neutral fat, that no doubt is left that practically all the fat in the lacteals of the mesentery is present as neutral fat, and only a small fraction as fatty acid. The determination of free fatty acid was made by evaporating the ethereal extract to dryness, dissolving in hot alcohol, and titrating with deci-normal sodic hydrate solution, using phenol-phthaleïn as an indicator.

The neutral alcoholic solution thus obtained was next evaporated almost to dryness, a measured volume of standard alcoholic potash (approximately $\frac{N}{2}$) was then added, and the mixture boiled for twenty to thirty minutes; the flask in which the boiling took place being fitted with a reflux tube. The contents were then neutralized with semi-normal hydrochloric acid, and the difference between the amount of acid required and that necessary to neutralize the volume of alcoholic potash originally

added, gave the necessary datum for calculating the amount of neutral fat which had been saponified by the alkali.[1]

Experiment 1. In this preliminary experiment only a small amount of chyle was collected (0·65 c.c.), which contained 0·0198 grammes of ethereal extractive. But even this small amount was enough to demonstrate that the amount of free fatty acid present was relatively small, for on dissolving the ethereal residue in hot alcohol, and titrating as above described, it was found that only 0·1 c.c. of the decinormal alkali was sufficient to give a distinct pink with phenol-phthaleïn. Now, theoretically, the volume of decinormal alkali required, on the assumption that the ethereal extract consisted entirely of free fatty acid, would be 0·7 c.c.; hence less than one-seventh of the extract was free fatty acid.

Another indication of the presence of neutral fat and not fatty acid, which was also seen in the other experiments of this series, was the difficulty with which the ethereal residue dissolved in the alcohol. Neutral fat only dissolves in appreciable quantity when the temperature of the alcohol is near boiling point, while free fatty acid, such as oleic acid, dissolves appreciably even in cold alcohol. Hence, if a small quantity, such as a single drop, of olein be warmed with a considerable quantity of alcohol (50 c.c.), it remains visible as a round globule until the alcohol is almost boiling. On the other hand, a much greater amount of fatty acid dissolves before the temperature of the alcohol is much raised. This qualitative sign is of considerable value in determining whether one is dealing with an extract consisting chiefly of neutral fat, or of fatty acid. In the series of experiments on digestion of soap with tissue extracts, it was found, in contra-distinction to those in the present series, that the ethereal extracts dissolved in the alcohol with great ease.

Experiment 2. A dog, weighing approximately 12 kilogrammes, was fed with 100 grammes of olive oil at 9.30 a.m. Anaesthetized at 3.30 p.m., exposed lacteals, and filled 17 drawn-out tubes with chyle, by cutting open lacteals. Weight of chyle = 1·2990 gramme; weight of total ethereal extract = 0·0618 gramme; weight of free fatty acid, calculated from direct titration = 0·0028 gramme; weight of neutral fat, calculated from alkali required for saponification = 0·0564 gramme.

Expressed as percentages of the total fatty matter, the neutral fat forms 95·3 per cent., and the fatty acid 4·7 per cent.

Experiment 3. A dog weighing 8·6 kilogrammes was fed with 50 grammes of olive oil at 9.30 a.m. At 4.30 p.m. (interval seven hours), the animal was anaesthetized with chloroform and ether, and 23 drawn-out tubes were filled with fatty chyle from the mesenteric lacteals, in which the amounts of fatty acid and neutral fat were estimated as before, with the following results:—

Weight of lymph	=	0·9550 gramme.
,, ethereal extract	=	0·1052 ,,

1. The above is Kottstorfer's method of estimating the amount of neutral fat (see Sutton's *Volumetric Analysis*, 8th edition, p. 402); preliminary experiments with pure neutral olein gave practically theoretical results.

Weight of fatty acid	=	0·0042 gramme.
„ neutral fat (as olein)	=	0·1031 „
Percentage of neutral fat	=	96·1
„ fatty acid	=	3·9

Experiment 4. A dog, weighing 12·4 kilogrammes, was fed with 100 grammes of olive oil at 9 a.m., and the fatty chyle was collected at 3 p.m. (interval six hours), by means of a pipette drawn out to a capillary end, from the mesenteric surface after puncture of the lacteals, into a small porcelain dish. The lymph was weighed and analysed as before, with the following results:—

Weight of lymph	=	1·8712 gramme.
„ ethereal extract	=	0·1450 „
„ fatty acid	=	0·0042 „
„ neutral fat (as olein)	=	0·1326 „
Percentage of neutral fat	=	96·9
„ fatty acid	=	3·1

Accordingly, the fatty matter present in the mesenteric lacteal vessels is proven to consist practically all (upwards of 95 per cent.) of neutral fat, demonstrating that the synthesis from the products of digestion occurs in the intestinal wall.

Experiments on the Nature of the Fatty Constituents in the Intestinal Mucosa during Fat Absorption

Immediately after the collection of the lymph used in the preceding series of experiments, the animals were killed, and the entire small intestine was removed, cut open longitudinally, and thoroughly washed with water to remove adherent matter. The intestine was then laid out with the mucous surface upward on a glass plate, and the mucosa rubbed off with the back of a knife. The scraped off mucous membrane was weighed and extracted first with a mixture of alcohol and ether (1 alcohol to 3 of ether), and then with ether alone. The solvents were decanted off, the solutions mixed, and evaporated to dryness. The dry residue was next extracted with dry ether, filtered, and the ethereal extract evaporated to dryness. The residue was finally weighed, and the amount of fatty acid and fat estimated.

Experiment 1. Total weight of moist mucous membrane = 31·7 grammes; weight of total ethereal extract = 1·1524 gramme; weight of fatty acid by titration = 0·1802 gramme; weight of neutral fat by difference = 0·9722 gramme; relative percentages of neutral fat and fatty acid = 84·3 and 15·7.

Experiment 2. Weight of moist mucosa = 14·4 grammes; weight of total ethereal extract = 0·8074 gramme; weight of fatty acid, by titration = 0·2904 gramme; weight of neutral fat, by saponification and titration = 0·5303 gramme; relative percentage of neutral fat and fatty acid = 64·6 and 35·4.

These figures show that the greater part of the fatty material present in the mucosa is in the form of neutral fat, but that the percentage of free fatty acid is much greater than that in the lymph collected from the mesenteric lacteals (see previous series of experiments), showing that the process of transformation in the cells is in progress and incomplete.

The results can, however, only be taken as approximate, since it is impossible, even with the most careful washing, to be certain that all the adherent, unabsorbed and unchanged fat is washed out from between the villi.

Experiments on the Action of Pancreatic, Lymphatic, and Intestinal Tissues and of Cell-free Extracts Prepared from these Tissues upon Solutions of Soap and Glycerine

These experiments were commenced with the view of determining whether the synthesis of neutral fat, described by Ewald, was due to the action of an enzyme contained in the intestinal cells, which might be separable from them, or whether such a synthesis occurred only in presence of the surviving, though isolated, cell.

Hence the first experiments were conducted only with filtered and centrifugalized extracts, but negative results having solely been obtained with such extracts (so far as such synthetic action was concerned), similar experiments were also instituted with extracts containing cells, and in addition, in order to obtain information as to the action of all types of cell involved in fat absorption upon soap and glycerine solutions, comparative experiments were carried out with cells and extracts of the pancreas and abdominal lymphatic glands.

The tissues and extracts used were prepared from glands of the cat, dog, ox, or pig, and similar effects were in all cases obtained.

In the case of the intestinal mucosa, the intestine taken from a freshly killed animal was cut open longitudinally from end to end, and then thoroughly washed either in a stream of running tap water or with 0·75 per cent. solution of sodium chloride. In the earlier experiments saline was employed, but in later experiments, in which water was subsequently to be used as an extractive agent, water was used for preliminary washing out, and as this was found not to cause any alterations in the property of the extracts, in the later experiments water was always used to wash the surface, even when saline was to be employed later as an extractive agent. It may be stated here that no difference was ever found throughout the series of experiments in the action of extracts made, on the one hand, with distilled water, and on the other hand with saline solutions containing 0·75 per cent. of sodium chloride.

The mucous membrane, after thorough washing, was scraped off from the intestine in lengths of three to four inches at a time by laying it flat on a glass plate and scraping lengthwise with the back of a table knife. In this way a soft semifluid

mass was obtained, which was gathered into a heap on the glass plate and chopped with the knife. It was then transferred to a mortar and rubbed up either alone or mixed with fine sand. Portions were then weighed out and extracted with appropriate quantities of the various extractives for varying times in an incubator at 36° C.

In those cases where the action of the cells was to be tested, the ingredients to be acted upon were added in weighed quantities before this first period of digestion in the incubator. In some cases chloroform was added as a preservative, but in others, to prevent any paralyzing action upon the cells, this re-agent was left out.

In the cases where the action of extracts only of the cells was to be tested, the tissue treated as above described was allowed to undergo digestion for a variable period, in presence of chloroform,[1] to prevent bacterial growth. The extract was then filtered, afterwards thoroughly centrifugalized, and the clear extract used for the experiments. In the case of the pancreas and abdominal lymphatic glands, the tissue was first finely minced and subsequently treated in similar fashion to the intestinal mucosa.

The strength of the extract employed was varied in the different experiments, and the strength is stated in each case. The soap used was sodium oleate, which was prepared from pure olive oil. The oleic acid obtained by hydrolysis of this soap had a melting point of 17·5° C., and 0·214 gramme required 7·6 c.c. of $\frac{N}{10}$ caustic soda for neutralization, the theoretical amount being 7·57 c.c.

Series I

Experiment 1. The small intestine of a cat in which digestion, chiefly of bread, was going on, but no fat was visible in the lacteals, was treated as above described, saline being used as the washing fluid.

A quantity of about 20 grammes of mucosa was obtained, and digested with four times its volume of normal saline for ninety hours in an incubator at 33° C. The extract was then filtered and centrifugalized, when a perfectly clear, slightly yellow, solution was obtained.

Ten cubic centimetres of this fluid were taken in a test tube, and, after the addition of 0·2 gramme of sodium oleate and 0·052 gramme of glycerine, the mixture was placed in a bath at a temperature of 38·5° C. The test tube was agitated after it had attained the temperature of the bath until all the oleate added had dissolved to a clear solution.

After an interval of one hour a few oily globules were visible under the microscope.

Next morning, an interval of seventeen hours thirty minutes having elapsed since the commencement of the experiment, the fluid was yellow and cloudy, like an

1. To this end, also, the saline, or water, used as an extractive was sterilised by previously boiling and allowing to cool before use. This was found to be a very useful precaution.

emulsion, and some drops of material insoluble in the fluid were found floating on the surface, while under the low power of the microscope a large number of oily globules of varying size were visible.

Experiment 2. The abdominal lymphatic glands of the same cat, which was used for experiment 1, were taken, and likewise digested at 33° C. for ninety hours in presence of chloroform, with four times their weight of normal saline. The extract was filtered and centrifugalized, giving a clear reddish-yellow fluid in which no cellular elements were visible under the microscope.

Ten cubic centimetres of this fluid were taken, 0·2 gramme of sodium oleate, and 0·052 gramme of glycerine were added, and the clear fluid obtained on warming to 38·5° C. and agitating, was digested in a bath at 38·5° C.

The experiment was started at 6 p.m., and next morning, at 10.30 a.m., (interval = 16h. 30m.) there was a thick layer of yellow-coloured oil on the surface which formed a temporary emulsion on shaking, and the fluid gave under the low power a field filled with globules, closely resembling milk as seen under the microscope.

The strength of the lymphatic gland extract in this particular experiment, judging from the depth of the oily layer, was much greater than that of the extract of intestinal mucosa, but later experiments showed that no general law to this effect applied.

The oily layer here formed looked so like olive oil, and the appearance of the globules under the microscope so closely resembled that of fat globules as seen in milk, that it was at first thought that olein had been synthesized from the sodium oleate and glycerine added to the extract ; but a determination of the amount of free fatty acid in the oil soon dispelled this illusion.

The contents of the test tube were extracted by shaking four times with an equal volume of ether. In this process, it was observed not only that the oily layer disappeared on the first addition of ether, but also in the shaking up with this first portion of ether, the aqueous layer became quite clear. The four portions of ether, after separation from the aqueous layer, were united, the ether was evaporated off, and the residue weighed (wt. = 0·1188 gramme). The residue was then dissolved in warm alcohol, and the solution was titrated with deci-normal sodic hydrate, using rosolic acid as an indicator, 3·7 c.c. were required for neutralization, indicating 0·1040 gramme of oleic acid. Hence 87·5 per cent. of the soap decomposed by the extract had gone to form oleic acid and not neutral fat.

It might be thought that the development of oleic acid here occurring was due to acidity of the lymphatic extract employed, but a portion of the same lymphatic extract, which had, therefore, also been ninety hours in the incubator, was tested with rosolic acid and found to be alkaline, the alkalinity being equivalent to $\frac{N}{60}$ of sodic hydrate.

Hence, if the setting free of the oleic acid be due to an acid formed in the lymphatic extract, that formation only occurs in the presence of the soap, and is a concomitant of the action upon the soap.

Experiment 3. The production of acid from soap is not stopped by the prolonged action of sulphuretted hydrogen[1] upon the tissue cells or extracts, as is shown by the following taken from a number of experiments.

The abdominal lymphatic glands of an ox, obtained fresh from the abattoir, were cleaned free of fat, minced finely and extracted with five times their weight of distilled water in an incubator kept at 36° C. for a period of ninety-two hours.

Before the flask containing the cells was placed in the incubator, it was thoroughly saturated with sulphuretted hydrogen gas and tightly corked. At the end of the interval, the contents of the flask still had a strong smell of sulphuretted hydrogen. The fluid was now filtered from the tissue elements, and a water-clear extract of a greenish-brown colour was obtained. This was charged again with sulphuretted hydrogen gas, corked, and allowed to stand for ten days at room temperature; it was then filtered from a slight deposit of sulphur, and found on testing to be slightly acid; acidity $= \frac{12N}{50}$. The fluid was hence made slightly alkaline by addition of excess of sodium carbonate, alkalinity $= \frac{N}{60}$ to rosolic acid as indicator.

A portion, measuring 40 c.c. of the alkaline extract, which had been thus subjected to treatment by sulphuretted hydrogen, was taken, and to it were added 0.8 gramme of sodium oleate, and 0·4 gramme glycerine. The flask containing the mixture was then saturated with sulphuretted hydrogen, corked, and placed in an incubator at 36° C. After twenty-four hours digestion at this temperature, an oily layer had appeared on the surface of the flask, and a single extraction with ether gave a residue weighing 0·355 gramme, and containing 0·341 gramme of free oleic acid.

Experiment 4. In three cats, in which the stomachs and intestines were empty of food, the pancreas and small intestines were removed, prepared as before, and digested with normal saline for a period of forty-three hours in an incubator at 36° C. The solutions were made distinctly alkaline at the end of the period to phenolphthaleïn, and the strengths were then made equal to one in nine of the fresh tissue.

Each extract was then divided into four portions of 25 c.c. each, which had substances added to them, and were treated as follows:—

No. 1. 25 c.c. extract + 0·5 gramme oleate + 0·1 gramme dextrose + 0·16 gramme glycerine.

No. 2. 25 c.c. extract + 0·5 gramme oleate + 0·16 gramme glycerine.

No. 3. 25 c.c. extract + 0·5 gramme oleate.

No. 4. 25 c.c. extract + 0·5 gramme oleate + boiling before digestion.

1. These experiments were devised to imitate the reducing condition present in the intestine, and so favour the synthesis of neutral fat, they were carried out with pancreas and intestinal mucosa, in addition to lymphatic glands, as above described, and in all cases gave negative results as regards fat synthesis and positive as regards acid formation from soap.

After this procedure, the eight tubes were placed in a water bath at 36° C., and examined at intervals.

Intestine. Examined one-and-a-half hours later, all four of the intestinal tubes showed turbidity, and, under the microscope, thickly studded fields of oily globules.

Examined after the lapse of nineteen hours thirty-five minutes, all four of the intestinal extracts show a thick creamy layer on top, a little less marked on the boiled tube, No. 4, than on the others, but still very obvious. Under the microscope all four show fields crowded with oil globules. The reaction of the contents of all four tubes is still distinctly alkaline to phenol-phthalëin.

Pancreas. The four tubes containing pancreatic extract examined one hour after the commencement of the digestion show in each case a milky fluid, and the microscope demonstrates abundance of oily globules. All four tubes give an alkaline reaction to phenol-phthalëin, but less strongly marked than in the case of the intestine. The action obviously is more intense here than with the intestinal extracts. Examined at the end of eighteen hours, there is a thick layer of oil and cream at the top of each tube, which is not apparently any less in the boiled tube than in the other three tubes.

Microscopic examination shows in all four tubes oily globules of all sizes, and in great number. There are apparently no fewer globules in No. 4 than in any of the other tubes.

The four pancreatic tubes are all still alkaline to phenol-phthalëin.

The eight tubes were then extracted with ether, and the weight of ethereal extractive, and amount of free fatty acid determined as above described ; the results are given in the following table :—

	Contents of tubes	Weight of ethereal extract	Weight of free oleic acid	Percentage of free oleic acid
Intestinal Mucosa	No. 1. Oleate + glycerine + dextrose ...	0·1800[1]	0·1664	92·4
	No. 2. Oleate + glycerine	0·1258	0·1256	99·8
	No. 3. Oleate alone	0·1582	0·1466	92·7
	No. 4. Oleate alone, then boiled... ...	0·1322	0·1269	96·0
Pancreas	No. 1. Oleate + glycerine + dextrose ..	0·2688	0·2580	96·7
	No. 2. Oleate + glycerine	0·2568	0·2312	90·0
	No. 3. Oleate alone	0·2176	0·2140	98·3
	No. 4. Oleate alone, then boiled... ...	0·2394	0·2284	95·4

1. The weighings were taken to ½ of a milligramme.

The results of this experiment shows that the formation of free acids is greater throughout in the case of pancreatic tissue than in that of the intestinal mucosa. The presence of dextrose apparently increases slightly the amount of free acid liberated, but has no action in producing any synthesis of neutral fat.

Experiment 5. Since it might be supposed that in the preceding experiments the soap was hydrolysed by the water or saline used as a solvent, and not by any active constituent of the tissues extracted, a series of controls was carried out in which the same percentage of soap was dissolved, as follows :—

No. 1. Distilled water, 40 c.c. + sodium oleate, 0·8 gramme + glycerine, 0·4 gramme.

No. 2. Normal saline (0·75 per cent.), 40 c.c. + sodium oleate, 0·8 gramme, + glycerine, 0·4 gramme.

No. 3. Solution of Na_2Co_3 (0·2 per cent.) 40 c.c. + saturation with carbon-dioxide + 0·8 gramme of sodium oleate + 0·4 gramme of glycerine.

No. 4. Oxalated pig's plasma, 40 c.c. + sodium oleate, 0·8 gramme + glycerine, 0·4 gramme.

All four flasks containing these respective solutions were placed in the incubator at 36° C.

Examined as soon as they had attained the temperature of the incubator, No. 1 was found to be completely dissolved to a clear solution ; Nos. 2 and 3 were opalescent and contained a good deal of undissolved oleate ; No. 4 was clear, but contained a small amount of undissolved oleate.

Examined four hours later, the appearance presented to the eye was much the same as at the previous examination, while microscopic examination showed no trace of fat globules in any of the four, merely fine amorphous granules were present in Nos. 2 and 3.

Examined forty-six hours after the commencement of the experiment, during all of which interval the four flasks had been kept at a temperature of 36° C., Nos. 1 and 4 were found to be perfectly clear solutions without a trace of cloudiness or precipitation, and showing a clear field under the microscope. Nos. 2 and 3 were opalescent and contained a sediment, but when examined by the microscope showed no trace of oily globules, and there was no surface layer of oil.

Hence these controls clearly demonstrate that the formation of oleic acid observed in the previous experiments was due to some hydrolytic agent present in the extracts of the tissues.

SERIES 2

In the previous series of experiments, extracts free from cells were employed, and attention was now turned to the products obtained when the cells were present, along with the same constituents as in the previous series.

Also, to make the experiments comparable with those of previous workers, the solutions, after digestion, were evaporated down to dryness before extraction and then extracted with dry ether. Moreover, to equalize, as far as possible, the amount of soap dissolved by the ether, controls were employed in which an equal percentage of soap was added after evaporating the control (to which no soap had been previously added) to dryness. This was the method followed by HAMBURGER, who, however, did not add a definite weighed quantity of soap to the controls, but merely an approximately equal quantity.

By this method the quantity of total ethereal extract is largely increased, but the control experiments, as well as the determinations of Series 3 (*vide infra*), showed that the increase was due to dissolved soap and not to neutral fat.

Experiment 1. The intestinal mucosa of a cat was prepared as usual, and two quantities of 6 grammes each were weighed out. To portion No. 1, 30 c.c. of normal saline, 0·6 gramme of sodium oleate, and 0·3 gramme of glycerine were added; and to portion No. 2, 30 c.c. of normal saline only were added.

The two flasks were placed in the incubator for a period of forty-three hours at 36° C., and were then taken out and the contents evaporated to dryness in porcelain basins on a steam bath.

When both were dry, 0·6 gramme of sodium oleate was added to No. 2, and then in each case four extractions were made with ether. The ethereal extracts were united in each case, the ether was evaporated off, and the two residues were weighed. Each residue was then dissolved in warm alcohol, and the amount of free fatty acid determined by titration with $\frac{N}{10}$ alkali, using phenol-phthalëin as an indicator.

The results are given in the following table :—

	Weight of ethereal extract	Weight of free oleic acid	Difference	Percentage of free oleic acid
No. 1	0·6290	0·4399	0·1891	69·9
No. 2 (control)	0·3708	0·2510	0·1198	67·7

Here it is obvious that a considerably greater percentage of oleic acid is extracted in No. 1 than in No. 2, on account of the previous hydrolysis, but the difference between total extract and oleic acid, as shown in the third column, is nearly equal in the two cases, there being a difference of only 69 milligrammes. Further, this difference is found in the opposite direction in the succeeding experiment (*vide infra*), and hence lies within the limit of experimental error and does not indicate any formation of neutral fat.

The considerable amount of oleic acid obtained in the control (No. 2) of this experiment, at first sight appears to indicate that ether is capable of extracting oleic

acid from dry sodium oleate, this is not, however, the source of the oleic acid here obtained, as is seen from the controls in Experiment 1, Series 3 *(vide infra)*. The source of this oleic acid is the neutral fat of the mucosa itself.

Experiment 2. The abdominal lymphatics of the same animal used in Experiment 1 were finely minced, and treated like the intestinal mucosa in that experiment.

Portion No. 1 weighed 1·83 gramme, and was treated with 18 c.c. of normal saline, 0.36 gramme of sodium oleate, and 0·15 gramme of glycerine.

Portion No. 2 weighed 1·63 gramme, and was treated with 16 c.c. of normal saline only.

The two portions were digested in the incubator at 36° C. for a period of 115 hours; both were then evaporated to dryness; 0·32 gramme of sodium oleate was added to No. 2, and each was then extracted four times with ether, and the weights of total ethereal extracts and amounts of free fatty acid present were determined.

The results, calculated for convenience of comparison to two grammes of tissue, are given in the following table :—

	Weight of ethereal extract	Weight of free oleic acid	Difference	Percentage of free oleic acid
No. 1	0·4024	0·2751	0·1273	68·3[1]
No. 2 (control)	0·3782	0·1825	0·1957	48·2

Here the difference between total extract and free acid, as shown in the third column, is somewhat greater in the case of the control.

Series 3

In this series of experiments, extracts containing cells were used in some of the experiments, and cell-free extracts in others. In all cases, in addition to determining the free fatty acid, the amount of neutral fat was also determined afterwards by Köttstorfer's saponification method, and was always found very low. The figures given in the various experiments are intended to show how low the amount is, but do not quite accurately represent it, for the amount *if any*, present is quite too small for accurate determination. These figures are based on amounts of standard alkali of 0·05 to 0·25 c.c., and it is obvious that calculations based on such small quantities exceed the true values, since a distinct reaction with the indicator

1. The much lower percentage of oleic acid in this and the preceding experiment is due to the dry method of extraction, in which more soap is dissolved than when shaking up of the solvent with the aqueous extract is employed as a method of separation. The actual amount of oleic acid formed is, however, as great in these two experiments as in the others.

was obtained in each case. In other words, the figures merely show that any amount of neutral fat which may be formed under such conditions falls within the limit of experimental error.

In each experiment of this series, a division of the extract into four portions was employed. Portion No. 1 had 2 per cent. of sodium oleate, and 1 per cent. of glycerine added ; No. 2 had 2 per cent. of sodium oleate alone ; No. 3 had 2 per cent. of sodium oleate added after previous boiling ; and No. 4 had nothing added, and was not boiled.

A comparison of the results after digestion will hence show the effect, if any, of presence of glycerine as between Nos. 1 and 2 ; the effect of boiling upon the production of oleic acid, as between Nos. 2 and 3 ; while No. 4 gives the amount of fatty extractives and action thereon in the tissues or extracts alone.

The four portions, after the above preparation, were in all cases subjected to digestion for an equal, but variable, period, which is recorded under each experiment.

The four portions were next evaporated to dryness on a steam bath, and each dry residue was then treated by the following method.

The residue was extracted four times with ether ; the ether was separated off and evaporation to dryness gave the total ethereal extractive, which was weighed.

The ethereal residue was dissolved in warm alcohol, and titrated with decinormal sodic hydrate, using phenol-phthalëin as indicator, titrating rapidly and taking the first appearance of a pink tinge to indicate neutrality, so as to avoid saponification of any trace of neutral fat which might be present.

After neutralizing the free fatty acid, the bulk of the alcohol used as a solvent for the titration was evaporated off to prevent dilution of the standard alcoholic potash, now to be used for saponification.

The alcoholic potash employed for saponification was made up approximately of $\frac{N}{2}$ strength. As it very slowly changes in strength on standing it was titrated against standard $\frac{N}{2}$ hydrochloric acid, immediately before using for each experiment, and the result so obtained used for that experiment.

In each case 10 c.c. of the alcoholic potash was added and the mixture boiled in a flask, fitted with a reflux tube, for twenty minutes. The mixture was then titrated back to neutrality with standard $\frac{N}{2}$ hydrochloric acid. Thus if 10 c.c. of the alcoholic potash used required 9·6 c.c. of $\frac{N}{2}$ acid, and after boiling with the previously neutralized ethereal extract required 9·45 c.c., the amount of alkali used in saponifying any neutral fat present would be equivalent to 0·15 c.c. of $\frac{N}{2}$ hydrochloric acid.

Experiment 1. Intestinal cells (pig) in distilled water ; period of digestion, seventeen hours. In each portion 10 grammes of intestinal mucosa, 30 c.c. of distilled water ; to No. 1, added 0·8 gramme of sodium oleate and 0·4 gramme of glycerine ; to No. 2, 0·8 gramme of sodium oleate only ; to No. 3, 0·8 gramme of sodium oleate, after previous boiling ; to No. 4, nothing added and not boiled previous to digestion.

The results are given in the following table :—

	Total ethereal residue	Oleic acid	Olein	Soap, etc.
No. 1	0·8034	0·1942	0·0294	0·5798
No. 2	0·6168	0·1885	0·0368	0·3915
No. 3	0·5772	0·1294	0·0368	0·4110
No. 4	0·0526	0·0309	0·0211	0·0016

It is here obvious from column three that the olein present is exceedingly small, and when the amount present in the tissue itself, as shown in No. 4, is deducted, comes well within the limit of experimental error. Column four again demonstrates that the difference between total extract and free fatty acid found in all the previous experiments is due to dissolved soaps.

Addenda to this Experiment

No. 1. *Controls with distilled water.*—The effect of the processes used in the above experiment upon solutions in distilled water (*a*) of sodium oleate and glycerine, and (*b*) of sodium oleate alone, in identical strengths with those used in the experiment, was next tested, the same quantities of solution, period of digestion at the same temperature, and the same methods of extracting and titrating being employed throughout. These controls serve also for the succeeding experiments of this series. The results were as follows :—

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1 (oleate + glycerine)	0·6152	0·0704	0·0147	0·5301
No. 2 (oleate alone)	0·5746	0·0760	0·0294	0·4692

The results also show that the oleic acid formed in the case of the intestinal extracts was not due to the experimental procedures employed, and that the olein there found lies within the limit of experimental error.

No. 2. *Action of the intestinal extract after evaporation to dryness and extraction with ether.*—As experiment had shown that boiling for five minutes (in No. 3 of the previous experiment and elsewhere), although it somewhat diminished the power of the extract to produce oleic acid from sodium oleate, still left a considerable amount of activity untouched, the residue of No. 4, weighing 0·5786 gramme, to which no soap had been added, and which had not only been boiled but reduced to dryness and then extracted with ether so that it contained no fatty matter, was tested as to whether it still possessed any activity upon sodium oleate. For this purpose it was

extracted with 40 c.c. of distilled water, and 0·8 gramme of sodium oleate was now added, the mixture was digested at 36° C. for a period of seventeen hours, and then the determinations made as before with the following results :—

Total ethereal extract	Oleic acid	Olein	Soap
0·5352	0·2759	0·0147	0·2446

This experiment, therefore, shows that the substance causing the hydrolysis of soap into free fatty acid is not destroyed or removed by prolonged heating to boiling point, such as takes place in evaporating to dryness on a steam bath, nor by extraction with ether several times.

Experiment 2. Digestion with intestinal cells (pig) in distilled water for a shorter period. (Interval two-and-a-half hours).

The quantities taken, and experimental procedures were as in Experiment 1, but the time of digestion was reduced from seventeen to two-and-a-half hours, the results were as follows :—

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·5588	0·1664	0·0073	0·3851
No. 2	0·3956	0·1382	0·0147	0·2427
No. 3	0·5834	0·1382	0·0000	0·4452
No. 4	0·0432	0·0169	0·0294	0·0000[1]

The amounts of oleic acid here formed are almost as large as in the longer period of digestion, showing that equilibrium had practically been reached in the shorter interval of two-and-a-half hours.

Experiment 3. Intestinal cells (pig) in 0·75 per cent. saline, quantities and procedure as in previous experiment, interval two-and-a-half hours.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·4620	0·1269	0·0147	0·3204
No. 2	0·3224	0·1269	0·0073	0·1882
No. 3	0·2706	0·0733	0·0147	0·1826
No. 4	0·0224	0·0197	0·0147	0·0000[1]

1. It will be observed that oleic acid and olein slightly exceed total ethereal extract, this is due to experimental error.

Experiment 4. Clear intestinal extract (pig) in distilled water, one of gland to five of water, period of extraction = 44 hours, period of digestion = 2½ hours. Quantities taken and subsequent treatment as in previous experiments.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·3552	0·2171	0·0147	0·1234
No. 2	0·3338	0·2284	0·0220	0·0834
No. 3	0·3292	0·1946	0·0147	0·1199
No. 4	0·0302	0·0197	0·0220	0·0000

Experiment 5. Clear intestinal extract (pig) in 0·75 per cent. saline, one of gland to five of saline, period of extraction = 44 hours, period of digestion = 2½ hours.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·3252	0·1946	0·0073	0·1233
No. 2	0·3586	0·2171	0·0073	0·1342
No. 3	0·4774	0·2143	0·0147	0·2484
No. 4	0·0152	0·0085	0·0147	0·0000

Experiment 6. Pancreatic cells (pig) in 0·75 per cent. saline, one of gland to seven of saline, period of digestion = 15 hours, quantities and procedure as before.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·9330	0·6796	0·1323	0·1211
No. 2	0·9922	0·7360	0·1617	0·0945
No. 3	0·7518	0·2763	0·1941	0·2814
No. 4	0·3036	0·2058	0·0955	0·0023

The olein found in this experiment arises from the fat present in the pig's pancreas (compare Experiment 7); it is true that the amount in the control, No. 4, is somewhat less than that present in the other three, but this is probably due in great part to the protective action of the soap present in Nos. 1, 2, and 3, which is attacked

by the active substance of the gland. It may be noticed that the boiling of No. 3 diminishes the formation of oleic acid (No. 3, column 2) much more markedly than in the case of the intestinal and lymphatic extracts.

Experiment 7. Clear pancreatic extract (pig) in 0·75 per cent. saline ; one of gland to seven of saline ; period of extraction = 23 hours ; period of digestion = 16 hours.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·5344	0·4766	0·0073	0·0505
No. 2	0·4582	0·4484	0·0073	0·0025
No. 3	0·5212	0·4596	0·0073	0·0543
No. 4	0·0396	0·0282	0·0147	0·0000

Experiment 8. Abdominal lymphatic cells (pig) in 0·75 per cent. saline ; one of gland to seven of saline ; period of digestion = 15 hours.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·6738	0·2961	0·0073	0·3704
No. 2	0·5766	0·2933	0·0058	0·2775
No. 3	0·5062	0·1720	0·0080	0·3262
No. 4	0·0754	0·0296	0·0044	0·0414

Experiment 9. Clear extract of abdominal lymphatic glands (pig) in 0·75 per cent. saline ; one of gland to seven of saline ; period of extraction = 23 hours ; period of digestion = 16 hours.

	Total ethereal extract	Oleic acid	Olein	Soap
No. 1	0·5382	0·4174	0·0294	0·0914
No. 2	0·4472	0·3722	0·0147	0·0603
No. 3	0·4612	0·3638	0·0294	0·0680
No. 4	0·0266	0·0282	0·0147	0·0000

Discussion of the Synthetic Activity of Enzymes and of Living Cells

One of the most important results obtained from the work described above, is that neither cell-free extracts of the intestinal mucosa nor detached cells are capable of synthesizing neutral fats from their constituents; the positive results in this direction obtained by previous workers being shown to be due to incomplete analysis of the products obtained. On the other hand, it is quite as clearly demonstrated, by the experiments on the nature of the fatty constituents contained in the mesenteric lymphatic vessels during fat absorption, that all the neutral fat decomposed during digestion is resynthesized during the process of absorption back to neutral fat.

This result is in full accordance with the facts known regarding cellular activity as contrasted with enzymic activity throughout the body generally, and naturally leads the mind to enquire what are the usual respective limits of enzymic and cellular activity, and to a contrast between the types of chemical reaction induced respectively by isolated enzymes and by living cells.

It is at the present time becoming increasingly fashionable to attribute all the chemical processes occurring in living cells to the presence in the cell substance of soluble enzymes, which, if we possessed sufficient knowledge of the necessary manipulation, might be satisfactorily isolated, and made to perform their work apart from their natural laboratory, the cell; so that if all the contained enzymes had been isolated in common solution, the chemical changes occurring in the cell would be capable of being brought about by that solution.

There exists, however, no sufficient experimental basis for such a generalization; there is no proof, in the first place, that all chemical changes in the cell are due to enzymic action; nor, in the second place, even admitting that the process is carried out by a number of enzymes acting in consort, that the presence of the living protoplasm is not essential for correlating the work of the enzymes concerned, and regulating the extent of activity of each, so that a number of complex chemical reactions may be combined in such a fashion as to yield definite end-products in definite proportions. When a comparison is made of the products elaborated by the living cells of the body, and those produced by the activity of enzymes, certain very important differences become apparent, which may here be reviewed.

In the first place, the type of reaction induced by enzymes is very simple compared with that brought about by cells. The vast majority of enzymic reactions are simple hydrolyses, such as can readily be imitated by dilute acids or alkalies; in other cases there is merely a simple molecular rearrangement, or at most, the detachment of the elements of water and carbon-dioxide, as in the case of the yeast enzyme. On the other hand, when the activity of living protoplasm is called into play, there occurs a building up of most complex chemical products from simple constituents, such as has only been imitated in a few of the less complex instances in the chemical laboratory, and then

under conditions of temperature and presence of reagents such as cannot occur in the living cell, and hence by methods entirely different, and probably with types of energy different from those utilized in the living cell.

If the more complex operations occuring in the living cell were due to the action of enzymes, there is no apparent reason why such enzymes should not be equally obtainable alongside the enzymes which induce the simpler chemical changes. No such enzymes have, however, hitherto been obtained from living cells, and hence the view that such processes are due to enzymes has no experimental basis, and room is certainly left for the alternate hypothesis that these complex syntheses, which, *under the given conditions*, are peculiar to living matter, are brought about by the presence of the living protoplasm, and possibly by the development of a type of energy peculiar to living matter, and capable of acting, in presence of the cell which plays the part of an energy-transformer, upon the substances present in the cell and causing chemical transformations which give rise to the complex syntheses under discussion.

A second point of distinction between enzymic action and cellular activity is, that no instance is known in which a markedly endo-thermic reaction is capable of being induced by an enzyme, while there are numberless examples of such reactions being brought about by the action of living cells.

This is a characteristic difference of fundamental importance, whatever the explanation of it may be, and points to a much more complicated machinery for the induction of chemical action in the living cell than that which is present in the case of the enzyme. It is obvious that the energy for such endo-thermic reactions cannot come from the small amount of cell protoplasm, and hence it follows that the cell, in virtue of its structure, or of some intermediate type of energy connected with its structure, as a living organism, is capable of acting as an energy-transformer. The form of external energy so transformed may be very varied, it may be light energy, as in the case of the chlorophyll-containing cells of growing plants, or surplus chemical energy, as in the transformation of carbohydrate into fat by the connective tissue cell of the animal body. Even where only chemical sources of energy are involved, the action of the living cell is different from that of a single enzyme, in that energy which is obtained from the oxidation and breaking up of one portion of a substance can be stored in some fashion and utilized for the building up of other substances containing more chemical energy than that from which they were formed. Such a complex activity proceeding upon like substances in opposite directions at the same time has not been recorded in the case of an enzyme, and indicates a complexity of action in the case of living cells which is absent in enzymic action. Thus, when from a supply of carbohydrate food to a living cell, fat is formed, the process must consist in the oxidation of a part of the carbohydrate, and the energy so set free, instead of passing out of the cell as heat, must be utilized by the cell to yield the energy necessary for the reduction and combination of other molecules of the

carbohydrate to form fat. No enzyme is known which can utilize such a supply of energy to form fat from carbohydrate, and hence it seems probable that the living cell is capable of developing an intermediate form of energy, which then acts upon the portion of carbohydrate to be changed into fat, and yields to this the necessary energy for the processes of reduction and combination required for the production of fat.

Such a view of a type of energy peculiar to living matter is widely different from the old view of a vital force or vital energy of a mystic character, and with no relationship to the forms of energy found in non-living matter.

To prevent confusion, the term vital energy ought not to be used to represent it, the term *biotic energy* is here suggested, to indicate that from a definite amount of energy of any given type, such as solar energy or chemical energy, a type of energy may be produced which is capable of developing other forms of energy in the cell, such as mechanical or thermal energy, or the energy necessary for the production of endo-thermic reactions, and the evolution thereby of the complex products of organic synthesis manufactured by the cell.

It cannot be too strongly urged that the production of urea, or the sugars or other complex organic substances by laboratory reactions, which were at one time believed to be capable of production only by the cell, cannot be used in any sense as a proof of the non-existence of such a type of energy as has been enunciated above, and styled biotic energy.

Even had every individual substance formed under usual conditions in nature been synthesized in the chemical laboratory, no proof would thereby be given that such substances are formed in the living cell from the identical types of energy, or by the same intermediate processes, as they are formed experimentally in the laboratory.

In proof of the above statement, it may be urged that the same chemical substance may be formed synthetically by the use of different forms of energy ; in one case say by thermal energy, in another by electrical energy, and yet in another by radiant energy. If these are to be regarded as distinct types of energy, so also must the energy of the living cell, which carries out operations inexplicable by the application of the laws of diffusion and osmosis to the cell.

The fact that in the living cell there exists a mechanism for energy transformations dissimilar in structure and properties from anything found in inorganic or non-living nature, and producing results which cannot be imitated *under similar conditions* by any other form of energy-transformer acting only with non-biotic forms of energy, points strongly to the conclusion that the cell develops a type of energy peculiar to itself, with its own easily recognized criteria, which distinguish it from other forms of energy, and which is interchangeable in definite proportions with and for these other forms of energy. A form of energy as full of mystery as to its nature and properties as any of the non-biotic forms, but no more mystical than any one of them, and no

less strongly characterized and differentiated from them than they are from one another.

Whether it be granted or not that the living cell intermediately develops a type of energy peculiar to itself, the experimental fact remains that the cell is capable of inducing actions of greater complexity and involving more markedly endo-thermic reactions than the soluble ferment.

Thus the reaction induced by the enzyme is of a simple type, either a single substance is resolved into two or more substances, or by an inverse change two or more simple substances are united to form a single substance ; there are no intercurrent reactions of different type involved. But in the case of the cell, several reactions take place concurrently, and the energy set free from one reaction is used in running another, so that the whole process becomes complex and of a character, as to the nature of products formed, which is quite different from that due to an enzyme.

In one important respect, however, the action of the living cell and the enzyme are identical, that is, that neither the living matter of the cell nor the substance of the enzyme enters permanently into the chemical reaction induced. The quantities of matter transposed from one chemical form to another are disproportionately large, compared to the masses of cell or enzyme responsible for the chemical change, and hence, both cell and enzyme must be regarded essentially as energy-transformers for chemical energy.

The fact that enzymes are incapable of inducing markedly endo-thermic reactions is due to the fact, insisted upon above, that the reaction is simple in type, and hence an enzyme cannot oxidize part of the material, and utilize the supply of energy so obtained for the formation, by an inverse process, of a substance containing more chemical energy per unit weight than the original substance.

An enzyme can, however, under suitable conditions of concentration of the solution and temperature, induce synthetic change by altering the level of the equilibrium between the various types of energy interacting in the system.

Such synthetic changes due to enzymes occur, however, only in the case of reactions in which there is little change of chemical energy, as shown by the equality of heats of formation, within the limits of experimental error, of the substances involved in the reaction.

No synthesis due to enzymic action has been shown to occur where the heat of combustion of the substance formed in the synthesis is measurably greater than those of the substances from which it is formed.

All such reactions belong typically to the class of reactions known as *reversible reactions*, and in them the amount of chemical energy involved in the reaction is small compared to the masses of the reacting substances, or to the thermal capacities of those masses.

In such reactions the equilibrium point is that stage in the reaction at which there is equilibrium between the potential factors of the forms of energy (*viz.*, osmotic, thermal, and chemical) involved in the reaction.

In any given reaction the greater the difference in chemical potential, as measured by difference in heat of formation of the substances present at either end of the reaction, the more completely will the reaction run towards that side at which the chemical energy is at a minimum, and the greater will be the velocity of reaction.

In all reactions which are induced or accelerated by enzymes, the change in chemical energy is small, and it is for this reason that all such enzymic changes as digestion of proteids and hydrolysis of fats, starches, and sugars run with measurable velocities, and are, under certain conditions, reversible.

The function of the enzyme in accelerating such reactions or starting them when in complete abeyance, consists in increasing, in some manner, the difference in chemical potential in the solution.

It is only by an alteration in the difference in chemical potential that a reaction proceeding, with given concentration at a given temperature, can be altered in velocity; and in certain cases, even where the reaction when once started is markedly exo-thermic, the difference in chemical potential must be increased before the reaction starts.

The need for such an increase in the difference of the chemical potential is still more obvious when the amount of energy set free in the reaction is small, as in all cases of enzymic action.

Hence, in a great many cases, the action remains stationary until the enzyme is added.

Also different enzymes cause varying amounts of alteration in chemical potential, and hence the reaction stops at different stages with different enzymes.

Thus in the hydrolysis of starch to glucose, one enzyme stops at the stage of maltose, while another is capable of causing a sufficient amount of change of chemical potential to carry the hydrolysis on into glucose.

The above view of catalysis as a change due to alteration in chemical potential in a chemically reacting system, differs in certain respects from that enunciated by OSTWALD,[1] and now usually accepted.

According to OSTWALD, the action of a catalyser is merely that of accelerating (or retarding) a reaction which is already proceeding, it may be at an immeasurably slow rate, the catalyser itself not being altered in the reaction or adding energy to it, so that the point of equilibrium cannot be altered by the presence of the catalyser.

In other words, a catalyser merely acts upon the velocity of reaction, but cannot alter the end result or start a reaction which would not occur, provided a sufficiently long time interval were allowed to elapse, without its presence.

The view stated in this paper, however, is that a catalyser or enzyme may not only alter the velocity of a reaction, but may start a reaction which would not proceed at all in its absence, and may further alter the point of equilibrium of the reaction.

1. *Über Katalyse. Vortrag gehalten auf der 73 Naturforscherversammlung zu Hamburg*, 1901. Leipzig, S. Hirzel, 1902.

It may first be pointed out that the supposed thermodynamic proof that the point of equilibrium of a reaction cannot be disturbed by a body which is not itself altered in the reaction is erroneous, since the proof given excludes without adequate grounds the view that the catalyser, when present, may alter, without itself being permanently changed, the distribution of energy between the other constituents by altering the equilibrium of potentials of the thermal volume (osmotic) and chemical potentials of the system.

The thermodynamic proof above-mentioned consists in instituting a cycle in which, supposing that the point of equilibrium is not the same in the presence and in the absence of the catalyser, the substance with the higher amount of chemical energy is formed in the presence of the enzyme ; the enzyme is then supposed to be removed, and the excess of substance with higher chemical energy ; and then the enzyme may be once again employed for forming a fresh quantity of substance of higher chemical energy from the substance of lower chemical energy. In this manner, chemical energy may be perpetually formed without expenditure of energy, since the enzyme is not changed in the process.

The fallacy of the reasoning lies in the fact that the energy employed in the formation of chemical energy comes from other forms of energy present in the solution, such, *e.g.*, as volume or osmotic energy, by a redistribution of potentials due to the presence of the catalyser in the solution, and that immediately on removal of the catalyser in the second stage of the imaginary cycle, the system will revert to its old position by an inverse distribution of potentials, so that the substance formed in the presence of the catalyser is reconverted back in the absence of the catalyser into the substance from which it was formed, accompanied by an inverse flow of energy. There is hence no theoretical basis for the view that the equilibrium point must be the same in a chemical system in the presence and in the absence of a catalyser. That an enzyme cannot start a reaction which will not proceed in its absence is merely a particular case of the above, and hence similar reasoning may be applied to it.

Under ordinary conditions, the synthesis of a substance of higher chemical energy from one of lower chemical energy can only occur, either when the difference in energy is small and can be taken from the volume energy (osmotic energy) of the solution, *e.g.*, as in the synthesis of maltose from dextrose in concentrated solution ; or when the catalyser is supplied with some form of energy extraneous to the system ; as in the synthesis of organic plant substance by chlorophyll-containing cells from solar energy, or by fat-forming cells by the use of a degradation of the chemical energy of a part of the system. For a higher form of energy such as chemical energy cannot be formed from heat energy at the same temperature. Hence usually, the work of a catalyser consists in starting, or in accelerating, a reaction which runs exo-thermically with degradation of chemical energy and evolution of heat.

Experimental results also support the view that catalysers are capable of starting

chemical reactions as well as accelerating them, and further, that the extent of action depends upon the power of the catalyser.

Thus a sterile solution of starch remains indefinitely unchanged in measurable degree, but in presence of ptyalin or maltase is rapidly converted into dextrin and maltose, where the hydrolysing action ceases. On the other hand, dilute acid or invertin carries the process on a stage further into glucose.

Coagulated white of egg remains for days and weeks unchanged in presence of 0·2 per cent. of hydrochloric acid, or in 0·5 per cent. of sodic carbonate, but if pepsin or trypsin be added to these fluids respectively, it is dissolved completely in a few hours, and the extent and nature of the change varies with the particular enzyme added. Casein solution undergoes no slow spontaneous coagulation in the absence of its appropriate ferment, but coagulates in a few minutes when it is present. All these facts, and many similar ones, argue strongly for the view that an enzyme not merely alters the velocity of the reaction, but also that it is responsible for its inception, and determines its amount and character.

Summary

1. Absorbed fat is re-synthesized to neutral fat in the intestinal mucous membrane, as shown by the large percentage of neutral fat in ethereal extracts of the mucous membrane taken during fat absorption, and by the fact that *all* the fat in the lymph of the mesenteric lacteals during fat absorption, before the abdominal lymphatic glands are reached, is neutral fat.

2. The detached cells of the pancreas, mucous membrane of intestine, and mesenteric lymphatic glands have a powerful action in setting fatty acid free from soap, and a similar action is obtained with cell-free extracts of such tissues; but *no synthesis of neutral fat has been obtained by the action of such cells or extracts upon solutions containing soap and glycerine.*

3. A comparison of results 1 and 2 leads to the conclusion that the living cell is capable of inducing syntheses which are not brought about by enzymes, and has led to a discussion of the relative properties of enzymes and cells in which the following conclusions are drawn :—

(*a*) The type of reaction is much more complex in the case of the living cell than that of the enzyme.

(*b*) The living cell can perform endo-thermic syntheses by causing, at the same time, oxidation and reduction processes in one and the same chemical substance.

(*c*) Such forms of energy-transformation as occur in the cell are peculiar in type, and are best explained on the assumption that a form of energy, termed in this paper *biotic energy*, is developed by the living cell.

(*d*) An enzyme probably produces its effect by causing alterations in chemical potential, and as a result can not only vary the velocity of a reaction, but can also start it or leave it at rest at various stages; and, provided the amount of energy transference from osmotic to chemical energy is not great, may cause a considerable amount of synthesis and a variable point of equilibrium.

OBSERVATIONS ON THE PHYSIOLOGY OF THE CEREBRAL CORTEX OF THE ANTHROPOID APES

OBSERVATIONS ON THE PHYSIOLOGY OF THE CEREBRAL CORTEX OF THE ANTHROPOID APES

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SINCE presenting our former note on this subject, we have obtained some further observations, though the number is still less than we should wish, owing to the rarity and expense of the material. Our further observations have been upon five chimpanzees of the commoner variety, and upon one more orang.

The statements given in our former communication have been confirmed in all respects by our observations obtained since then. We can, further, now make the following statements in addition :—

The whole of the surface of the 'island of Reil' has proved 'inexcitable' under faradization, with currents even considerably more intense than those sufficing to excite muscular movements when applied to the precentral convolution. This is noteworthy, because the large extent of the insula is a character distinguishing the brain of the anthropoid apes from that of the lower apes, and bringing it nearer toward the human type.

Faradization of the cortex of the inferior frontal convolution in either hemisphere has failed in our hands so far to elicit movements of any satisfactory degree of regularity or constancy ; and this even under use of currents much stronger than those which suffice when applied to the 'motor' cortex proper. The movements for which, in particular, careful search was made, were those connected with vocalization. From the posterior region of the convolution, at scattered points, and without constancy even at them, strong faradization occasionally seemed to induce movements in the larynx, distinguishable from the rhythmic of respiratory origin. Judging from such evidence as we altogether obtained, we conclude that either (1) no Broca 'speech centre,' at all distinctly foreshadowing the human, exists in these anthropoid brains, or (2) that direct faradization of the Broca speech cortex is inefficient itself to evoke vocalization. These two inferences, are, of course, not mutually exclusive, and both the suppositions may be correct.

Repeated observations on excitation of the cortex of the precentral convolution, confirm an opinion we had formed at the time of our former communication, and indicated in the diagram then furnished, but that we did not verbally express. This is to the effect that the anterior limit of the 'motor' field is not of sharp, abrupt character, but fades off forward somewhat gradually. This edge extends further forward under '*Bahnung*.' Under general conditions producing lowered excitability of the cortex it retires backward in the direction of the central fissure.

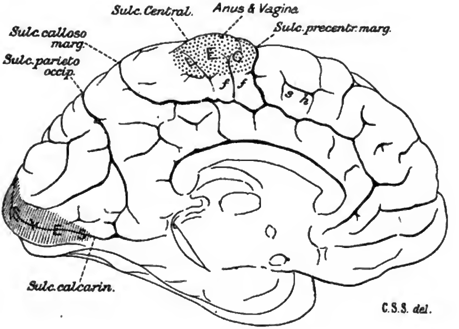
In a similar manner the boundary of the area for any particular movement may by '*Bahnung*' be extended beyond its average limit. The special form of movement provoked from a given spot of cortex is thus influenced by the particular forms of movement excited from neighbouring points just antecedently.

Among movements elicited from the cortex of the 'facial' region, we have in two instances seen protrusion of the tongue, succeeded by forcible closure of the jaws following rapidly before retraction had withdrawn the tongue behind the arcades of the teeth ; so that in these instances the tongue was caught by the closure of the teeth. This sequence of movements presents interest, as evidencing that a sequence of movements evoked by excitation of the cortex may exhibit in some respects faulty co-ordination. The movement is also of interest as a result of direct cortical excitation, which harmonizes with the biting of the tongue in epileptic seizures.

Ablation of the facial area of the 'motor' region was performed in one individual. A crossed hemiparesis ensued in the lips, cheek, tongue, nasal fold, and lower eyelid (very slight), but not in the upper lid, eyebrow, or frontal region.

As to the recovery of movement that occurs in a limb rendered paretic by ablation of its cortical area in the 'motor' region, we find the following points: If all the area, which when faradized evokes movements of fingers, thumb, and wrist primarily, and not as a later sequel to movements starting elsewhere, be excised, the paretic condition of the hand which ensues is severe, but rapidly diminishes. In a few weeks the hand is again very fairly and freely used. If, then, the whole of the corresponding area in the opposite hemisphere is removed, a similar paresis similarly ensues in the other hand, and runs a similar course ; but this second lesion does not produce, so far as we have been able to discover, the slightest recrudescence of the paresis already recovered from in the first hand. On the contrary, the first hand is almost at once employed more freely and successfully than prior to the second operation, presumably because of greater inducement to use it during the disability existing in the second hand. If, then, later, after the second hand has regained its use, the remaining part of the arm area first operated upon be ablated, this causes no obvious recrudescence of paresis either in the first hand or in the second hand. It causes severe paresis at shoulder, and to some extent at elbow, on the side crossed to the lesion, but this, again, is in great part of temporary character, and is largely recovered

from. In accord with the absence of recrudescence of the hand paresis on ablating in the third operation the remaining intact part of the arm area, we found that faradization of that part (elbow and shoulder) provoked, as usual, movements of elbow and shoulder, but not of hand itself, or only of hand late in a general arm movement, and that very rarely. In short, neither the ablation or excitation methods gave any evidence that the remaining part of the arm area had taken on the functions of the ablated hand area. The recovery of the hand movement seems, therefore, not due to either the adjacent cortex of the same hemisphere, or the corresponding hand area of the cortex of the opposite hemisphere, taking on the functions of the ablated cortical hand area.



DESCRIPTION OF FIGURE

Brain of a chimpanzee (*Troglodytes niger*). Left hemisphere ; mesial surface. The extent of the 'motor' area on the free surface of the hemisphere is indicated by the black stippling. On the stippled area, 'LEG,' indicates that movements of the lower limb are directly represented in all the regions of the 'motor' area visible from this aspect. Such mutual overlapping of the minuter sub-divisions exists in this area, that the diagram does not attempt to exhibit them. The pointing line from 'Anus, etc.,' indicates broadly the position of the area whence perineal movements are primarily elicitable.

Sulc. Central. = central fissure. *Sulc. calcarin.* = calcarine fissure. *Sulc. parieto occip.* = Parieto-occipital fissure. *Sulc. calloso marg.* = Calloso-marginal fissure. *Sulc. precentr. marg.* = Precentral marginal fissure.

The single italic letters mark spots whence, occasionally and irregularly, movements of the foot and leg (*f f*), of the shoulder and chest (*s*), and of the thumb and fingers (*h*) have been evoked by strong faradization. Similarly the shaded area marked 'EYES' indicates a field of free surface of cortex, which under faradization yields conjugate movements of the eyeballs. The conditions of obtainment of these reactions separates them from those characterizing the 'motor' area.

Faradization of the cortex of the post-central convolution, though not itself eliciting movement, when employed at certain places, facilitates the elicitation of movement by faradization at certain points at about the same horizontal level in the pre-central convolution. In other words, from certain parts of the post-central convolution, a facilitating influence (*bahnung*) can be exerted upon somewhat adjacent parts of the pre-central convolution.

Removal of the adjacent levels of the pre-central convolution does not render the post-central convolution 'excitable'; that is to say, destruction of the pre-central convolution does not make it the more possible to obtain movements under faradization from the post-central convolution.

The motor cortex of the infant chimpanzee, a few weeks old, is readily excitable by faradization. Its reactions do not appear to differ obviously in this respect from those obtainable from the adult. The movements it yields are not choreiform in character.

The spinal degeneration ensuing upon ablation of the arm area of the motor cortex of the chimpanzee, although it sometimes reveals a large uncrossed ventral pyramidal tract (direct Py. Tr.), does not do so in every case. Even after bilateral arm area lesions, the ventral pyramidal degeneration in the spinal cord may be very slight. The anthropoid cord resembles the human, therefore, not only in the possession of this tract, but in exhibiting in regard to it a remarkable degree of individual variation of development, as Flechsig showed to be the case in man.

The expenses of this research have been in part defrayed by a grant kindly allowed by the Scientific Grants' Committee of the British Medical Association.

THE ELECTRIC CONDUCTIVITY OF
MAMMALIAN NERVE

THE ELECTRIC CONDUCTIVITY OF MAMMALIAN NERVE

By R. S. WOODWORTH, Ph.D.
SENIOR DEMONSTRATOR OF PHYSIOLOGY, AND FORMERLY G. W. GARRETT FELLOW IN THE UNIVERSITY OF LIVERPOOL

IN continuation of the work of MACDONALD[1] on the physical properties of nerve, a series of measurements has been performed to determine the electric conductivity of the sciatic and ulnar nerves of the cat, and the influence on the conductivity and on the weight of a five minutes' immersion in saline solutions of different strengths. It will be recalled that MACDONALD found the injury current to be increased or decreased in a very definite way by immersion for five minutes in solutions of different concentrations. The strength of the injury current obeyed so definite a 'concentration law' as to warrant the conclusion that this current was produced by the nerve acting as a concentration cell; the more concentrated solution being in the interior of the nerve, that is to say, in the axis cylinder, and being separated from the less concentrated solution or lymph by the partially impermeable membranes surrounding the axis cylinder. In the hope that measurements of the conductivity of nerve as affected by immersion in saline solutions would further test this conception, the present series of measurements was undertaken. The author is indebted to MACDONALD for the suggestion of the problem and of methods as well as for the loan of apparatus. The latter, and also the procedure, were in the main the same as described by MACDONALD.

The sciatic and ulnar nerves were cut from a cat immediately after death, and kept in a moist chamber till each was used. Each nerve was cut to a length of about 5 c.m., its length, resistance, and weight were measured; it was then immersed for five minutes in a solution of potassium chloride of known strength, after which its length, resistance, and weight were again measured. From these data the specific conductivity, or conductivity per cubic centimeter, before and after immersion, were calculated.

The moist chamber in which the nerve was placed during the measurement of resistance, and also the solution in which it was immersed, were maintained as nearly as possible at 18° C., and correction was made for the slight deviations from this temperature.

1. *Thompson Yates Laboratories Report*, 1902, IV, part II, pp. 213-347.

Aside from minor series of experiments not here reported, one hundred and thirty-five sciatic and fifty-five ulnar nerves were immersed and measured. Each of the eleven strengths of solution was tried on at least ten sciatic nerves and four ulnar nerves. The number of cases and the uniformity of results are sufficient to guarantee the average determinations from large chance errors.

The lengthwise resistance of the sciatic nerve of the cat, when examined a few minutes after death, at 18° C., was found to average 200·7 ohms per cubic centimeter. The specific resistance of the ulnar nerve was somewhat smaller, 186·0 ohms on the average. Otherwise expressed, the specific conductivity of the sciatic nerve is 0·0050 mhos, and that of the ulnar nerve 0·0054 mhos. The substance of the ulnar nerve is thus a better conductor by about eight per cent. than the substance of the sciatic. This is not a chance result, but is almost invariable for every cat used, though a few exceptions occur in which the sciatic has as high conductivity as the ulnar.

The specific conductivity (lengthwise) of the posterior spinal roots is considerably less than that of the sciatic nerve, being about 0·003 mhos, and that of the cord is somewhat less still. GÖTHLIN[1] has found the specific conductivity of the corpus callosum to be much less than that of a peripheral nerve, and supposes[2] the difference to be due to the presence, in the sheath of the peripheral nerve, of well-conducting lymph spaces. If this were so, then freeing the peripheral nerve fibres of part of their envelopes and lymph spaces would increase the longitudinal specific resistance of the nerve. It is easy, in case of the sciatic, to pull out without violence one of the large strands of which the trunk is composed. I have done this with eleven nerves, and find the specific conductivity to be *increased*, not diminished. The specific conductivity of the strand is about one-eighth greater than that of the whole nerve. Now since the strand contains a larger proportion of nerve-substance proper than the whole trunk, the higher conductivity of the strand proves that nerve-substance proper is more conductive than the connective tissue envelopes with their lymph spaces. Moreover, in 'nerve-substance proper' is contained the non-conductive medullary sheath, as well as the axis cylinder, each occupying about half of the cross-section. The specific conductivity of the axis cylinder must, therefore, be more than twice as high as that of the outer envelopes with their lymph spaces. This result goes to substantiate MACDONALD's view that the interior of the nerve-fibre contains a more conductive, and therefore more concentrated, solutjon of electrolytes than the lymph bathing the outside of the fibres.

The high resistance of the posterior roots, and of the cord, is probably to be explained by the lack of parallelism of the fibres. It is known[3] that the

1. G. F. Göthlin. *Upsala Lakareforenings Forhandlingar*, 1902, VIII, 156, 163.
2. Göthlin, *op. cit.*, p. 165.
3. Tereg. *Archiv. für (Anat. u.) Physiol.* 1899, p. 319; Gothlin, *op. cit.*, p. 163.

resistance across the fibres is much greater than along them, and any structure other than a peripheral nerve, not having parallel fibres, would on that account have a relatively high longitudinal resistance. The ulnar nerve has a relatively small amount of connective tissue envelope and lymph space, and its higher conductivity may be due to this fact.

A few determinations of the specific gravity of a nerve, by use of glycerine solutions of different densities, have given the values 1·030 for the sciatic, 1·042 for the ulnar, and 1·045 for a separate strand of the sciatic. The values given above for specific conductivities should properly be multiplied by these numbers respectively, as the volume and specific conductivity were computed on the basis of a specific gravity equal to 1·000. The changes in specific gravity produced by five minutes' immersion in distilled water, and in a gramme-molecular solution of KCl, were about 0·005 in case of the sciatic nerve, and a little more in case of the ulnar.

The effects of immersion on the weight and on the electric conductivity of a nerve were found to be exceedingly definite functions of the strength of solution used. Weak solutions, while lowering the specific gravity, increase the absolute weight of a nerve; and concentrated solutions, while raising the specific gravity, decrease the absolute weight. This means that water diffuses from the weak solutions into the interior of the nerve, and from the interior of the nerve into strong solutions. The strength of solution that produces no change in the weight of the sciatic nerve is about one-eighth of the gramme-molecular solution (the latter being, for KCl, 7·45 per cent).[1]

The ratio of the weight after five minutes' immersion to the weight of the fresh nerve is as follows, in case of each strength of solution :—

Distilled water	1·033
1/40th gramme-molecular solution of KCl ...	1·017
1/20th " " " ...	1·015
1/10th " " " ...	1·006
1/8th " " " ...	0·996
0·15 " " " ...	0·993
1/5th " " " ...	0·972
1/4th " " " ...	0·953
1/2 " " " ...	0·948
1 " " " ...	0·928
2 " " " ...	0·901

The specific conductivity of a nerve is increased by immersion in strong solutions, and decreased by immersion in weak solutions. The concentration necessary in order to cause no change in the conductivity of the immersed nerve is

1. In case of the ulnar nerve, the strength of solution necessary to cause no change in weight was somewhat greater, according to my determinations, but there is here a slight source of error, due to the fact that the ulnar nerves were kept longer before being immersed. This error does not prejudice the values for the specific conductivity, for the latter was found not to change during over an hour's stay in the moist chamber.

about one-eighth of the gramme-molecular. This strength of KCl, or 0·93 per cent., is thus near to the isotonic solution of nerve, whether judged by weight or by conductivity.

The ratios of the specific conductivity after immersion to the original conductivity are as follows :—

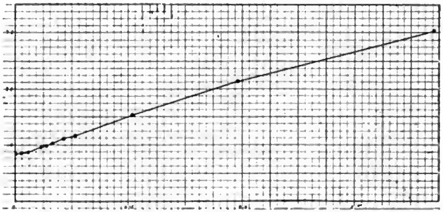
	Sciatic	Ulnar
Distilled water	0·831	0·766
1/40th gramme-molecular ...	0·847	0·811
1/20th „ „ ...	0·872	0·878
1/10th „ „ ...	0·948	0·899
1/8th „ „ ...	0·985	0·992
0·15 „ „ ...	1·033	1·020
1/5th „ „ ...	1·111	1·088
1/4th „ „ ...	1·163	1·172
1/2 „ „ ...	1·529	1·621
1 „ „ ...	2·137	1·972
2 „ „ ...	3·039	3·363

Five minutes' stay in distilled water does not deprive a nerve of all its conductivity, but of only about 20 per cent. Now MACDONALD[1] concluded, from the regularity with which the injury current of nerve obeyed the 'concentration law,' after five minutes' immersion in any solution, that this length of immersion sufficed to replace the lymph of the nerve sheathes by the solution used, without much altering the solution present in the axis cylinders. Adopting this conception, I infer that five minutes' stay in distilled water practically abolishes the conductivity of the external solution, so that the conductivity that remains belongs to the 'internal solution' in the axis cylinders. I thus infer that about 80 per cent. of the normal conductivity of a nerve is furnished by the internal solution.

This view is confirmed by the effects of other solutions on the conductivity. For if the internal solution remains the same after five minutes' immersion in any solution of KCl, while the external solution acquires in each case the concentration of the solution used, then the internal conductivity would remain constant throughout (= 80 per cent. of the normal conductivity of the whole nerve), and the external

1. *Op. cit.*, pp. 292-296.

conductivity would be proportional to the conductivity of the solution used. The whole conductivity of the nerve would be equal to the sum of a constant quantity *plus* a quantity proportional to the conductivity of the solution used. Now if the values given above for the conductivity after immersion (expressed as ratios to the conductivity before immersion) are plotted against the conductivities of the solutions, the result is as shown in the accompanying figure. The relation is a straight line, or very nearly so. Its equation would have the form $y = ax + c$. The ordinate (conductivity of the nerve after immersion) is equal to a constant c (80 per cent. of the normal conductivity of nerve) *plus* a quantity which is directly proportional to x, the conductivity of the solution. The whole series of results is therefore in harmony with the view that 80 per cent. of the conductivity of nerve is provided by the internal solution.



My measurements afford another means of calculating the relative conductivity of the internal and external solutions of a nerve. Before immersion the external solution has a conductivity about equal to that of the 1/8th gramme-molecular solution of KCl. After immersion the external solution has a conductivity equal to that of the KCl solution used; therefore, the conductivity of the external solution is changed in a known ratio. The original conductivity of the nerve is equal to the sum of the internal and external conductivities, while the conductivity after five minutes' immersion is equal to the same internal conductivity *plus* a known multiple of the original external conductivity. We have thus two equations for finding our two unknown conductivities—internal and external. MACDONALD[1] has already solved such equations in a typical case. Part of the object of my work was to see whether the values so calculated would agree in different cases and with different strengths of solution. I found considerable variability among the individual nerves, but close agreement between the averages for different strengths of immersing solution. The

1. *Op. cit.*, p. 342

results obtained with solutions close to the isotonic could not, however, be used for these calculations, because the error introduced by any slight variation in the concentration of the lymph was very great, and even led at times to negative values, here meaningless. Leaving out of account the solutions of 1/10th, 1/8th, and 0·15 gramme-molecular strength, the average results were as follows. The numbers give the fraction of the total conductivity that must be assigned to the internal solution :—

	Sciatic	Ulnar
Distilled water	0·831	0·766
1/40th gramme molecular ...	0·804	0·757
1/20th " " ...	0·775	0·781
1/5th " " ...	0·811	0·852
1/4th " " ...	0·832	0·822
1/2 " " ...	0·810	0·777
1 " " ...	0·819	0·841
2 " " ...	0·840	0·824
Average ...	0·815	0·801

This series of calculations agrees with the preceding one in assigning about 80 per cent. of the total conductivity of nerve to the internal solution. With this agrees also the rougher estimate based on the fact that a strand of the sciatic had a higher specific conductivity than the whole nerve. All the results are, therefore, in favour of MACDONALD's view that the internal solution of nerve is decidedly more concentrated than lymph.

ON THE DOSAGE OF THE MAMMALIAN HEART BY CHLOROFORM

ON THE DOSAGE OF THE MAMMALIAN HEART BY CHLOROFORM

By C. S. SHERRINGTON, F.R.S., and S. C. M. SOWTON

TO measure that dosage of chloroform under which the mammalian heart can and cannot continue to work efficiently was a problem given us by the Special Chloroform Committee of the British Medical Association.* Our preliminary Report was presented to the Committee on February 26 of this year. The present Report must be considered to deal still with the first stage of the enquiry.

Method

The method we have followed for perfusion of the heart has been that introduced by Langendorff[1] (1895). Our observations have dealt almost exclusively with the heart of the cat. We have on occasions used also the heart of the dog. In either case the heart was removed from the animal freshly killed.

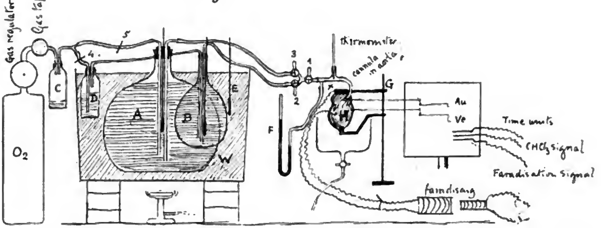
Into the aorta, close above its origin, a cannula was tied with its delivery end close above the sinuses of Valsalva, as practised by Newell Martin and Applegarth[2] (1890), and Langendorff[1] (1895). The other end of the cannula was connected with a tube leading from a reservoir containing a modified Ringer's solution (Fig. 1). This solution was kept warm in a water bath at 38° C. It was displaced from the flask containing it by introducing oxygen through it into the flask under a pressure 120 mm. Hg. above atmospheric. It had in the heart cannula a pressure of about 110 mm. Hg. The warm salt solution, after passing through the coronary system, collected in the right auricle and there dribbled over, keeping the whole heart moist and warm. The heart was held firmly by two clamps, one fixing the root of the lung, and thus indirectly the base of the heart, the other fixing the apex of the heart by the lower end of the left ventricle itself. A thermometer was let into the side of the cannula above the aorta.

The tube leading to the cannula was Y-shaped, and while one limb of the tube led from the reservoir of modified Ringer's solution, the other led from a similar though smaller reservoir, in which the modified Ringer's solution contained an accurately-measured quantity of chloroform. Both reservoirs were in the same water bath, and the fluid in each was similarly supplied with oxygen and under the

* The Committee consists of Dr. Barr, Dr. Dudley Buxton, Prof. Dunstan, Mr. A. Vernon Harcourt, Sir Victor Horsley, Dr. McCardie, Prof. Sherrington, and Dr. Waller (Chairman).

same pressure. Three 3-way taps in the Y-shaped tube allowed perfect control and interchange of the feeding fluids without any intermission of the supply to the heart. The capacity of the tubing between the $CHCl_3$ solution inlet and the heart was 5 cub. cent. The point marked on the graphic records by the $CHCl_3$ signal as the moment of commencement of admission of $CHCl_3$ solution to the heart is the moment at which this tap (2, Fig. 1) was turned, admitting the $CHCl_3$ solution to the delivery tube just above the aorta. The real moment of admission of the $CHCl_3$ solution to the heart was therefore a little later than that marked, beginning actually after the intervening 5 cub. cent. of unchloroformed fluid had passed into the heart. The rate of flow of fluid through the hearts employed was usually about 1 cub. cent. per second, the lost time therefore between the turning of the $CHCl_3$ tap and the real entrance of the $CHCl_3$ solution into the heart was therefore usually about five seconds.

Fig. 1



Disposition of apparatus. O_2, cylinder containing compressed oxygen. A, 10-litre flask containing modified RINGER's solution; B, 2-litre flask containing the modified RINGER's solution in which a known quantity of chloroform is dissolved; C, bottle preventing reflux of fluid into gas tap, etc.; D, bottle containing same chloroform solution as B; E, thermometer in the water-bath W; F, manometer, showing pressure of delivery of the solutions given to the aorta of the heart H; 1, 2, 3, three 3-way taps; 4, 5, clamps; Au, auricular recording lever; Ve, ventricular recording lever; X, diffuse pole of the unipolar faradization apparatus applied to the clamped tissue of the root of the lung; G, standard supporting the clamps which fix the isolated heart. The $CHCl_3$ signal records the moment of opening or closing of tap 2.

The contractions of the heart were recorded by attaching light writing levers to the auricle and ventricle, respectively. The threads were fixed to the heart by minute hooks made from the thin pins used by entomologists for pinning out small insects. The threads were in part of their length made of very narrow strips of thinnest gutta-percha sheet. This was found especially necessary for the auricular writing lever, the movement of contraction of the auricle being so quick as to otherwise lead to much deformation of the trace by the inertia of the lever. The levers

employed were straw, and were armed with writing points in the following way: A piece of capillary glass tubing was bent 90° at three places, and one arm of it inserted into the cylinder of the straw so as to lie as if in a longish groove along it. A little piece of very fine wire was attached to the lower free-hanging end of the pen, and could be bent with tweezers, so that, by altering the centre of gravity, the tip of the pen could be made to press in any desired degree upon the blackened travelling paper.

Electrodes upon a ball and socket joint were arranged so as to be applicable to the cardiac nerves. Arrangement was also made to faradize the heart wall. A diffuse electrode was fixed as a plate against the moist extra cardiac tissue above the base of the heart in the upper clamp; the condensed pole was formed by a minute hook inserted into the ventricle in its upper third; a very thin wire brought this hook into the circuit. In both cases the electrodes were fed by an inductorium served by a DANIELL cell; the moment of application of the faradizing current was indicated on the graphic record automatically by an electro-magnetic signal. Time, either in second or in five-second intervals, was likewise automatically recorded.

The fluid flowing from the heart was collected and measured when desired.

The solution we employed throughout was the modification of RINGER's solution devised by Dr. LOCKE.[1] We should probably never have attempted to work upon the isolated mammalian heart had we not witnessed, at the International Congress of Physiologists at Turin, two years ago, Dr. LOCKE's admirably successful demonstration of the effect of glucose upon the ventricular beat of the isolated rabbit heart. We therefore adopted his modification of RINGER's salt solution with some confidence that it might succeed in keeping active the heart of the cat, isolated under appropriate conditions. The cat is with us a much cheaper object of study than the rabbit, hence, since we desired a considerable number of observations, we preferred to employ it. The modified RINGER's solution has, in fact, fully answered our expectations. We have not added glucose to it, preferring for our initial observations to use the simplest solution possible. It, without the glucose, suffices to support, if duly oxygenated, the activity of the cat's heart for the greater part of a working laboratory day. We refer to the solution as 'modified RINGER's' in the present Report, because by 'LOCKE's solution' might be understood a glucose containing saline solution; but, as a fact, it is Dr. LOCKE's solution that we have invariably used, omitting only from it the glucose. LOCKE's modified RINGER's solution has the following composition[1]:—NaCl, 0·9 per cent.; $CaCl_2$, 0·024 per cent.; KCl, 0·042 per cent.; $NaHCO_3$, 0·01 per cent. in distilled water.

The amounts of chloroform that were measured in preparing the dilute solutions were sometimes small. In the earlier experiments, these, as well as the larger doses, were measured by volume at the room temperature 14°-16° centigrade. For these measurements we employed a burette divided into hundredths of a cub. centimetre, provided with a stop-cock, and armed for delivery with the capillary

needle of a hypodermic injection syringe. In the later experiments the quantities of chloroform have been measured by weight. The transference of the chloroform solutions from vessel to vessel under agitation in air we have endeavoured, as much as possible, to avoid. Also any unnecessary exposure of the vapour to india-rubber. The tubing leading from B (Fig. 1) to the tap 2 (Fig. 1) was composed, as far as possible, of glass; the cork of flask B was, however, of india-rubber. The oxygen gas introduced into flask B for expelling its fluid was in the later experiments led on its way to B through a layer of chloroform solution (in D, fig. 1) of the same dilution as that to be expelled from the reservoir B (Fig. 1). In these ways we tried to minimize the loss of chloroform that must to some extent occur from our prepared solution of it between its preparation and its actual entrance into the coronary vessels of the heart.

In regard to the quantities of fluid perfused, these have in our experiments been large but variable. The pressure of the fluid as supplied in our experiments has varied little from experiment to experiment, and in each individual experiment hardly at all; the pressures have throughout the series of experiments ranged from 90 mm. Hg., in some earlier, to 110 mm. Hg. in some of the earlier and in all the later experiments. It is possible that in some cases a portion of the fluid supplied has passed through incompetently acting aortic valve-flaps. The head of pressure being what it was, this, however, can have had no influence upon the coronary supply, except to make the readings of the quantities perfused through the coronary system larger than the real quantities perfused. The effect of this possibility upon the readings of the dosage as expressed—as we maintain it should not be expressed—by the mass of $CHCl_3$ administered to the heart in a given time, is to make our readings possibly higher than the quantities really administered to the cardiac muscle.

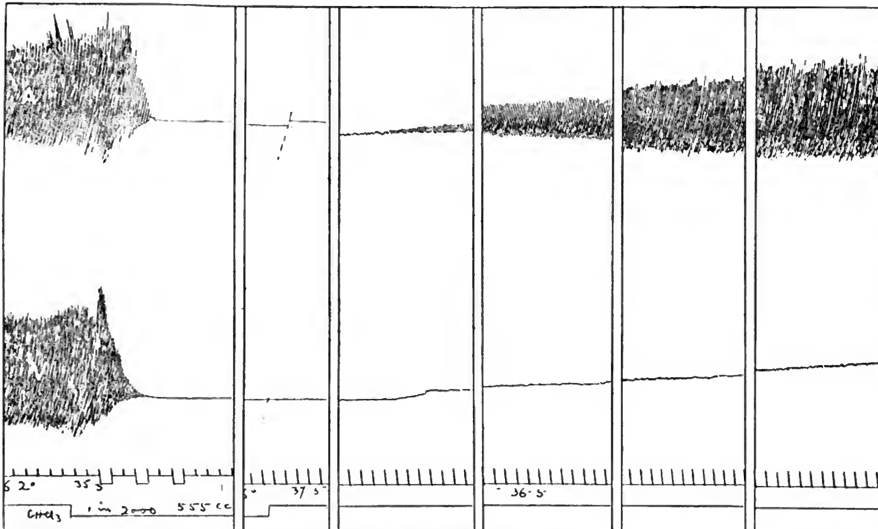
LITERATURE

The literature concerning the effect of chloroform on the heart is very large. We propose to reserve dealing with it until the occasion of our fuller report.

The perfusion of the isolated heart with fluids containing chloroform has been frequently practised on the isolated frog's heart. On the mammalian heart so perfused there is an isolated and incidental observation by Hedbom[4] in this paper on the action of drugs on the mammalian heart. But in his single observation the chloroform was added to blood already containing chloral, and was administered to a heart previously dosed with coffein; moreover, the dilution of the chloroform is not mentioned. At the time of our first communication to the Committee no other record than the above-mentioned by Hedbom had appeared. Since then, however, a preliminary communication 'On the action of chloroform, ether, alcohol, and acetone upon the excised mammalian heart'[5] has been made by Professor Tunnicliffe and Dr. Rosenheim to the Physiological Society.

RESULTS

The action of $CHCl_3$ on the heart, as studied in our experiments, may be described briefly separately for each degree of its dilution that we have employed.



Outset of Perfusion with $CHCl_3$. $CHCl_3$ discontinued. 3rd minute after discontinuance. 6th min. 8th min. 10th min.

FIG. 2

II.—Heart 22, Observation 4. Cat's Heart; $CHCl_3$ solution 0·05 per cent. in modified Ringer's solution, performed for five minutes five seconds.

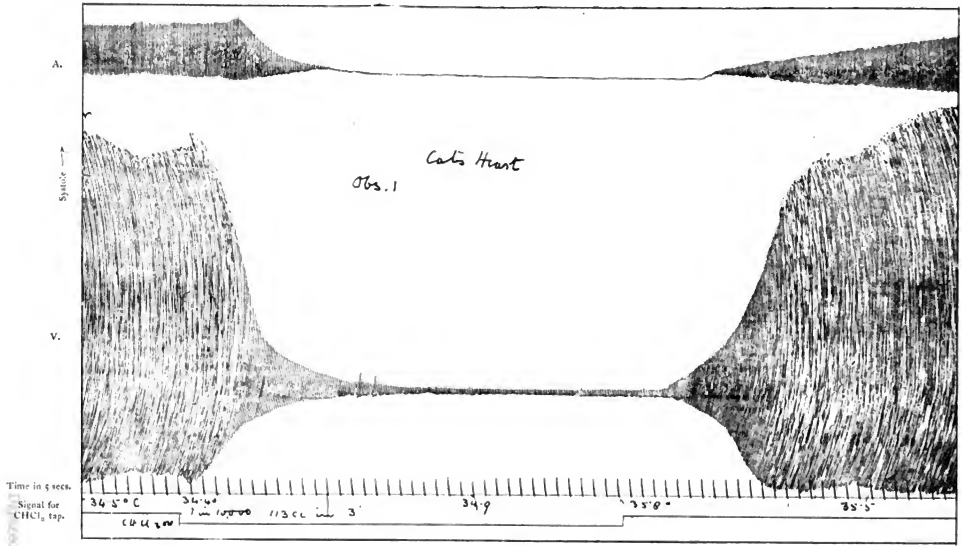


Fig. 3
IV.—Heart 24, Observation 1. Cat's Heart; $CHCl_3$ 0·01 per cent. solution in modified Ringer's fluid.

I. 1,500 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
($CHCl_3$ in ·15 per cent. dilution)

This solution administered for thirty seconds abolished, in less than twenty seconds, the beat both of auricle and ventricle. But, on replacing the chloroform solution by pure saline, the beat was subsequently completely recovered in both, in auricle slightly earlier than in ventricle. The ventricle recommenced beating about seventy-eight seconds after the $CHCl_3$ mixture had been discontinued; its recovery was abrupt in the sense that its very first beat was of considerable extent and power. The recovery of the auricular beat began more gradually.

Permanent abolition of the beat of the ventricle resulted from perfusion of this solution for sixty seconds; fibrillar contractions of the ventricle were all that subsequently were obtainable. But the auricle recommenced beating regularly seventy-five seconds after the end of the one minute's $CHCl_3$ perfusion, and was of normal extent and vigour 125 seconds later.

The resistance of the auricle-muscle to $CHCl_3$ in these high percentages appears much greater than is that of the ventricular. Perfusion with this ·001 solution for even sixty seconds, although completely and permanently annulling the ventricular beat, makes no permanent impression upon that of the auricle. The auricle began to show recovery about seventy-five seconds after cessation of the administrations of this $CHCl_3$ solution, whether the solution had been perfusing it for one, two, three, or five minutes, respectively.

II. 750 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
($CHCl_3$ in ·075 per cent. dilution)

This strength of solution almost immediately (in less than thirty seconds) extinguished the beat of both auricle and ventricle. As to subsequent recovery, in one heart both auricle and ventricle recovered completely subsequent to a continuous administration of the solution for five minutes; but the ventricle of the same heart completely succumbed to continuous administration of the solution for nine minutes, only fibrillar twitchings appearing in it subsequently. In another heart, administration of the same strength of solution for 305 seconds (Fig. 2) permanently destroyed the ventricular beat. In a third heart, the same strength of solution stopped the ventricular beat irrecoverably by one minute's perfusion, fibrillar twitchings being all that could be subsequently obtained.

In all these three hearts the auricle showed itself much more able to recover from $CHCl_3$ than did the ventricle. Even after nine minutes' perfusion with the solution the auricle recovered, although, to do so, it requires an interval of more than fifteen minutes' perfusion with the oxygented RINGER's solution.

With shorter periods of administration of the $CHCl_3$ solution, the auricle had usually been beating for fifty seconds to sixty seconds before the ventricle recommenced.

The return of beat of the ventricle was usually abrupt, suddenly bursting out in almost its full vigour. The beat of the ventricle was also often arrested five seconds to ten seconds earlier than that of the auricle.

III. 300 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·03 per cent. dilution*)

This strength of solution in every case practically extinguished the beat both of auricle and ventricle in thirty seconds from commencement of its administration to the heart. But complete recovery both of ventricle and auricle was obtained in every case. The recovery was complete and rapid, even after continuous exhibition of the drug for eighteen minutes. Two types of recovery seemed distinguishable. With $CHCl_3$ solutions of ·0001, or less, the beat recommences within about fifteen seconds from discontinuance of the administration. With the strength of solution now under consideration, namely, ·0002 $CHCl_3$, in one form of recovery, about seventy seconds elapse before any sign of beat, and full recovery is not reached until from three minutes to four minutes have elapsed, and there is merely a gradual return to, and not beyond, the force and excursion obtaining prior to the exhibition of the $CHCl_3$ solution. A second type of recovery is characterized by much speedier return to a strength of beat in excess of that originally obtaining. In this type the returning beat becomes obvious fifteen seconds to twenty seconds after cessation of the administration of the $CHCl_3$, and fifteen seconds to twenty seconds later the heart beats far more vigorously than before the chloroformization, showing periods of distinct acceleration as well as augmentation of beat. Some excitation of the augmentor-accelerator nerves of the heart is suggested. Those nerves, as is shown later in this report, are not paralysed by considerable depths of $CHCl_3$ action. This type of recovery seems to occur especially frequently after prolonged administration of $CHCl_3$.

IV. 150 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·015 per cent. dilution*)
(Figs. 3, 4 and 5)

With this strength of solution the amount of reduction of the activity of the auricle, as measured by reduction of the excursion of its recording lever, varied from 83 per cent. (reduction) to complete abolition (temporary). The mean reduction of the auricle beat was 96·9 per cent. for twenty-one observations on six hearts.

The mean reduction of the ventricle beat as similarly measured for the same six hearts was 97·6 per cent.

The full effect of the $CHCl_3$ solution was evident in about eighty seconds. Recovery was rapid and complete, both in auricle and in ventricle, in all cases. The beat began to improve within fifteen seconds of discontinuance of $CHCl_3$ perfusion, and recovery was complete about seventy-five seconds later. This held good even after prolonged perfusion, in one instance for more than ten minutes, in another for twenty minutes, continuous duration. (Fig. 5.)

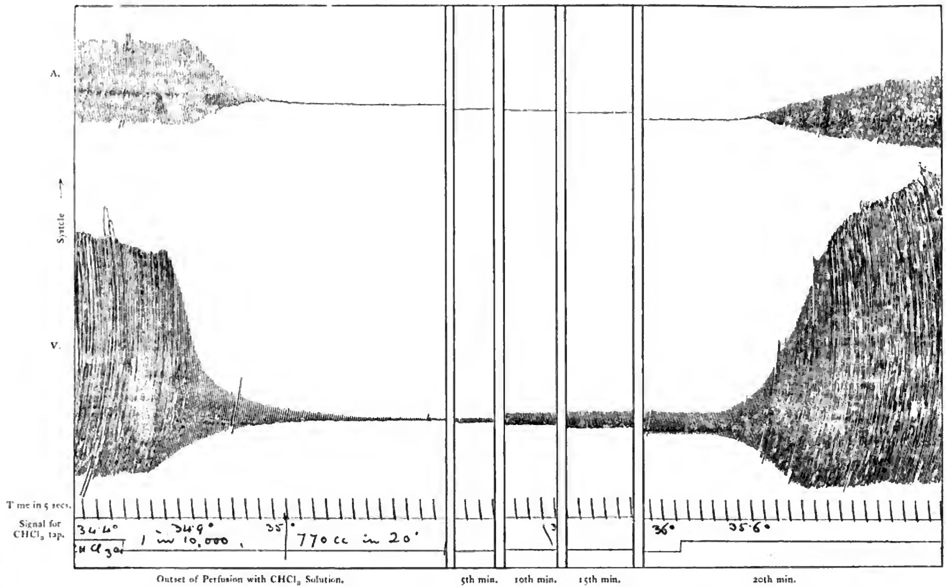


Fig. 4

IV.—Heart 24, Observation 3. Cat's Heart; $CHCl_3$ 150 mg. per Litre of modified Ringer's solution ($CHCl_3$ in 0·015 per cent. Solution), 770 c.cm. of the Solution passed through in 20 min.

PLATE VI (D)

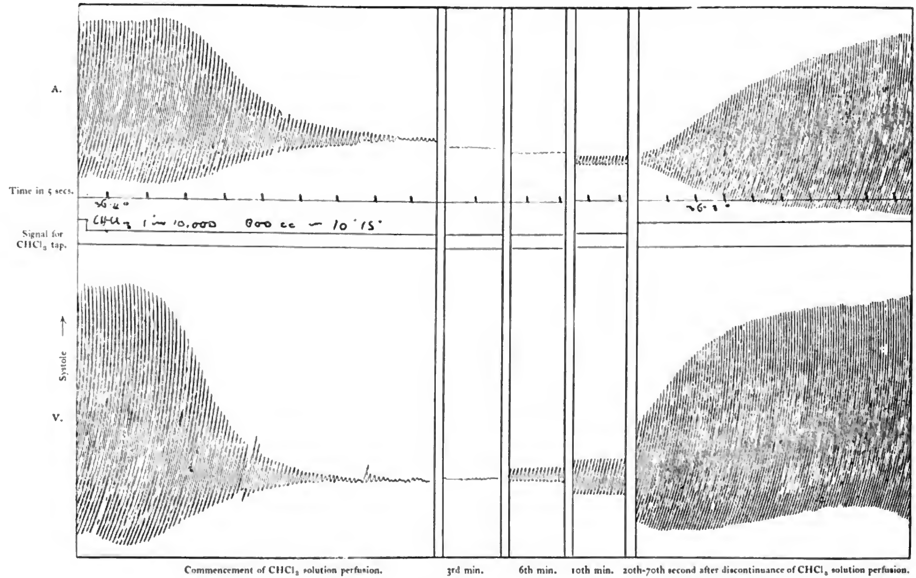


Fig. 5

IV.—Heart 26, Observation 4. Cat's Heart; CHCl$_3$ 0·01 per cent. in modified Ringer's solution; Temperature 36·4° C. at outset, 36·3° at end of observation. Perfusion with the Solution lasted ten minutes twenty seconds, in that period 800 c.cm. went through the coronary system.

The frequency and regularity of the beat was usually quite unaffected both in auricle and ventricle, even when the beat was reduced nearly to extinction.

V. 75 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·0075 per cent. dilution*)
(Fig. 6)

Under perfusion with this solution the amount of weakening of the auricle's beat varied in different observations between 64 per cent. reduction and practical abolition (temporary); the mean reduction in the cases observed was 79 per cent.

The mean reduction of the ventricle's beat was 86·7 per cent.

The solution usually took about fifty seconds to produce its full effect. Recovery was both in auricle and ventricle in all cases rapid and complete, even after prolonged perfusion with the solution, in one instance for twenty-two minutes forty seconds, during which time 1,800 cub. cent. of the $CHCl_3$ solution had flowed continuously through the coronary circulation. In this very instance both auricle and ventricle were beating fully normally within eighty seconds from the moment of discontinuance of the $CHCl_3$ solution.

The frequency of the rhythm of the beat remained quite unaltered by the $CHCl_3$ administration.

VI. 50 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·005 per cent. dilution*)
(Figs. 7 and 8)

Under this strength of $CHCl_3$ solution the weakening of the beat of the auricle varied from 30 per cent. reduction at outset of observations on Heart 20 to 89 per cent. reduction in the later observations made on Heart 21. The average reductions in Hearts 5, 6, and 21 were 71·1 per cent., 75 per cent. and 74 per cent., respectively, but in Heart 20 it was only 30 per cent. In the other hearts administration of the solution for forty seconds sufficed to cause a weakening of 50 per cent. The recovery of the auricle was always rapid and complete.

The weakening of the ventricle was in every case greater than that of the auricle, ranging from reduction of the amplitude of beat by 39 per cent. at outset of experiments on Heart 20 to extinction of the beat (temporarily) in Heart 5. In this latter heart, perfusion with the $CHCl_3$ solution for forty seconds sufficed to abolish (temporarily) the beat of the ventricle. But the ventricular beat nevertheless returned rapidly and completely after perfusion continued five times as long as that. Three-minute doses of this solution caused progressively rather more and more severe effects, as repeated, while the experiment proceeded.

The frequency of beat during the administration was never seen to alter from the rate obtaining when the chloroformization commenced.

VII. 38 mgrms. $CHCl_3$ per Litre Modified Ringer's Solution
($CHCl_3$ in ·0038 per cent. dilution)

Under perfusion of this strength of $CHCl_3$ solution through the coronary system the beat of the auricle suffered a reduction varying from 30 per cent. to 75 per cent. in five observations. The mean value of the reduction was 55 per cent. This estimate does not include the observations in Heart 19, an irregularly beating heart, in which the weakening by the solution amounted to certainly more than 70 per cent. In none of the hearts used was the auricular beat ever extinguished even temporarily by this solution.

The average weakening of the ventricular beat was 96 per cent., and it was usually extinguished altogether (temporarily).

Recovery was invariably rapid and complete, both in auricle and ventricle. Even when the perfusion of the $CHCl_3$ was continuously maintained for ten minutes the recovery on its discontinuance was prompt and complete. The heart thirty seconds after the discontinuance was beating as well as prior to the chloroformization.

When the exhibition of the drug in this strength of solution was prolonged it was noticed that the ventricle beat improved somewhat in the latter part of the administration upon what it was during the first few minutes of the perfusion; for instance, it would be visibly beating in the tenth minute, although without visible beat in the third minute.

VIII. 30 mgrms. $CHCl_3$ per Litre Modified Ringer's Solution
($CHCl_3$ in ·003 per cent. dilution)

Under perfusion of its coronary system, with this strength $CHCl_3$ solution, the mammalian heart showed a weakening of the auricular beat, as measured by amount of reduction of excursion of the recording lever, varying from 45 per cent. (Heart 29) to 85 per cent. (Heart 30). In one and the same heart it varied considerably, thus, from 28 per cent. at outset in Heart 28 to 62 per cent. later during the experiment. The reduction of amplitude of beat in twenty-one observations on five hearts averaged 66·8 per cent. The weakening did not occur so rapidly in the auricle as in the ventricle.

The degree of impairment of the ventricle's beat varied even more than that of the auricle, *i.e.*, from 15 per cent. to 95 per cent. The mean reduction in nineteen observations on the ventricles of four hearts was 91·7 per cent. The amount of reduction did not vary so widely in successive observations on the same individual heart as for observations on different hearts. The beat of the ventricle was in four observations (Heart 8) extinguished (temporarily) by this strength of $CHCl_3$ solution.

The duration of the dose with this solution was varied from forty-five seconds up to ten minutes. Sometimes weakening of the ventricle beat became

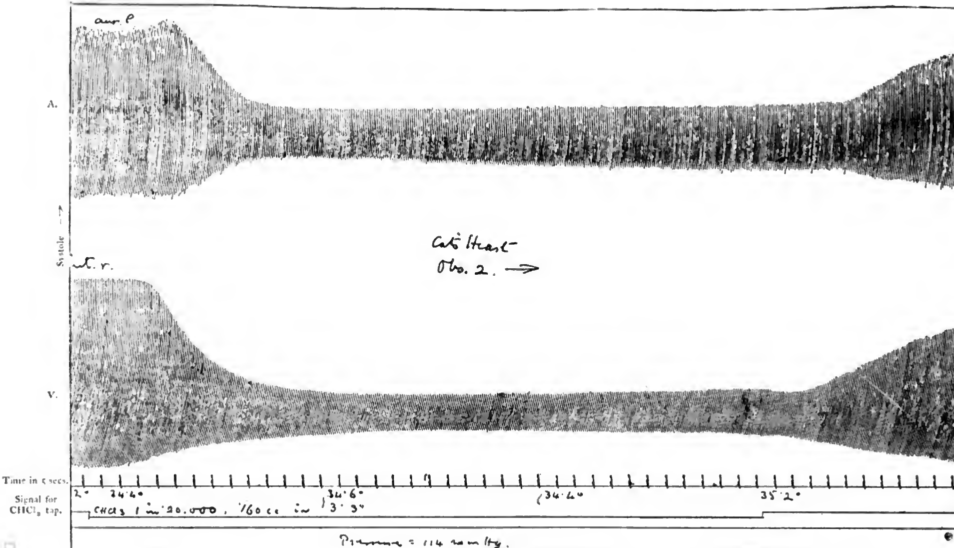


Fig. 6

V.—Heart 31, Observation 2. Cat's Heart perfused with $CHCl_3$, 1 vol. in 20,000 vols. modified Ringer's solution (0·0075 per cent. by weight) for three minutes three seconds; Pressure in Coronary Artery = 114 mm. Hg.

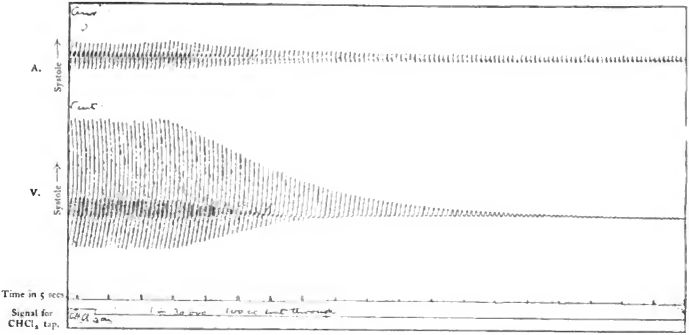


FIG. 7

VI.—Heart 5, Observation 6. Cat's heart; Perfusion of Coronary System with $CHCl_3$ 0.0032 per cent. in modified Ringer's solution; Temperature 38° C.; 100 c.cm., perfused in 125 seconds.

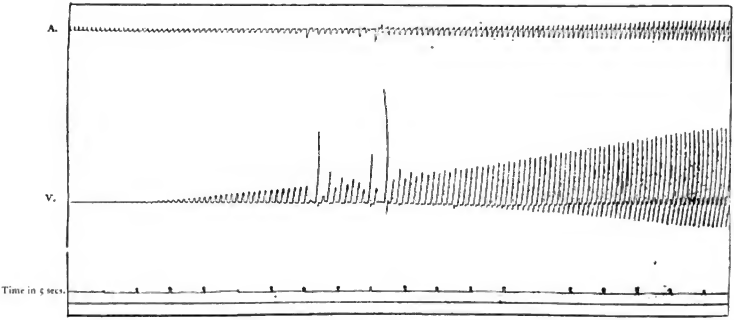


FIG. 7

VI.—Heart 5, Observation 6. Perfusion of the $CHCl_3$ solution discontinued; Recovery of Auricle and Ventricle.

obvious in five seconds from the admission of $CHCl_3$ fluid to the heart. The full effect was always reached in the second minute of administration, or sooner. The heart would not infrequently be beating better at end of the fifth or tenth minute than at end of the first minute, *e.g.*, Heart 28, observation 5. Even by end of the third minute of administration the beat is sometimes distinctly stronger than at end of the second minute.

Recovery was always rapid and complete, beginning a few seconds after cessation of the administration and becoming complete in two to four minutes.

The frequency of rate of beat remained unaffected by the exhibition of the $CHCl_3$ solution—thus, even when the beat was reduced in excursion by 95 per cent., the frequency remained absolutely the same. When, as was twice the case, the heart was beating in groups, the phasic groups continued unaltered in period, although each number of the series of contractions was reduced in the same proportion as were the other members. (Fig. 13).

IX. 20 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S FLUID
($CHCl_3$ in ·002 per cent. dilution)

This strength of chloroform solution we have employed in the instance of one heart only. The beat of the auricle was, as a mean of six observations, reduced in amplitude by 36 per cent.; that of the ventricle by 33 per cent. The cessation of exhibition of the drug, even when it had been perfused for five minutes continuously, was always followed by rapid and full recovery. The utmost (temporary) reduction of the auricle was 48 per cent., of the ventricle 50 per cent.; the least reduction of the auricle was 27 per cent., of the ventricle 28 per cent. The chloroform caused no alteration in the frequence or regularity of the rhythm of the beat.

X. 19 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
($CHCl_3$ in ·0019 per cent. dilution)

With this strength of solution we have observations on one heart only, a dog's heart. The beat of the auricle alone was recorded. The weakening of the beat, as estimated by the reduction of the excursion of the recording lever, amounted to 69 per cent. The full effect of the drug was obtained in about seventy-five seconds from commencement of the administration. When the administration was prolonged, *e.g.*, to ten minutes, the heart was rather less affected toward the end of the dose than at the beginning of the dose.

XI. 15 MGRMS. $CHCl_3$ PER LITRE MODIFIED RINGER'S SOLUTION
($CHCl_3$ in ·0015 per cent. dilution)
(Fig. 9)

The auricular beat was always obviously weakened. The degree of weakening, as indicated by reduction of the amplitude of the excursion of the recording

lever, varied between 10 per cent. and 50 per cent. The average weakening in seven observations on three hearts was 30·5 per cent. Recovery was always rapid, beginning a few seconds after cessation of the administration and reaching completion in from ninety seconds to four minutes.

The ventricular beat was likewise also obviously weakened. The reduction of the beat varied from 26 per cent. to 49 per cent.; it averaged in three hearts 38 per cent. The weakening began within ten seconds of commencement of administration, and obtained full effect in seventy seconds. It tended to diminish as time went on, being less marked at end of the seventh minute than at end of the second minute of administration.

In Heart 30 the effect of this strength of $CHCl_3$ solution was compared with that of a solution of twice the concentration on the same heart, the weaker solution being administered first. The weaker solution reduced the auricle beat by 30·3 per cent. (mean of three observations), the ventricle beat by 26 per cent.; the stronger solution reduced the auricle beat by 85·2 per cent. (mean of three observations). Recovery began about as soon after withdrawal of the stronger as after withdrawal of the weaker solution; but the completion of the recovery took longer for the former.

The frequency of rate of beat was not affected by the exhibition of the $CHCl_3$ solution.

XII. 10 mgrms. $CHCl_3$ per Litre Modified Ringer's Solution
($CHCl_3$ in ·001 per cent. dilution) (1 in 150,000 by volume)
(Fig. 10)

With this strength of solution, only a small reduction of the activity of either auricle or ventricle was observed. We have employed it in the case of two hearts. In ten observations on these two hearts the average reduction in amplitude of the excursion of the lever recording the ventricular beat was 21·7 per cent.; the average reduction of the auricular beat was more, but in these two hearts the auricular record was not very satisfactory. There was usually a very slight increase in amplitude of the ventricular beat, just at outset of the exhibition of the drug. Recovery was full, but not obviously more rapid than after stronger solution. There was no alteration in the frequence or regularity of the cardiac rhythm. An illustration of the effect of this strength of chloroform solution is given in Fig. 10.

With weaker solutions of chloroform than this last we have not worked.

Perfusions with Locke's Fluid

We have made comparatively few perfusions with Locke's fluid. The observations are at present insufficient to generalize upon. The fluid maintains the heart better than does Ringer's fluid, and as a vehicle for chloroform amounts from the physico-chemical point of view to practically the same thing as the 'Ringer.'

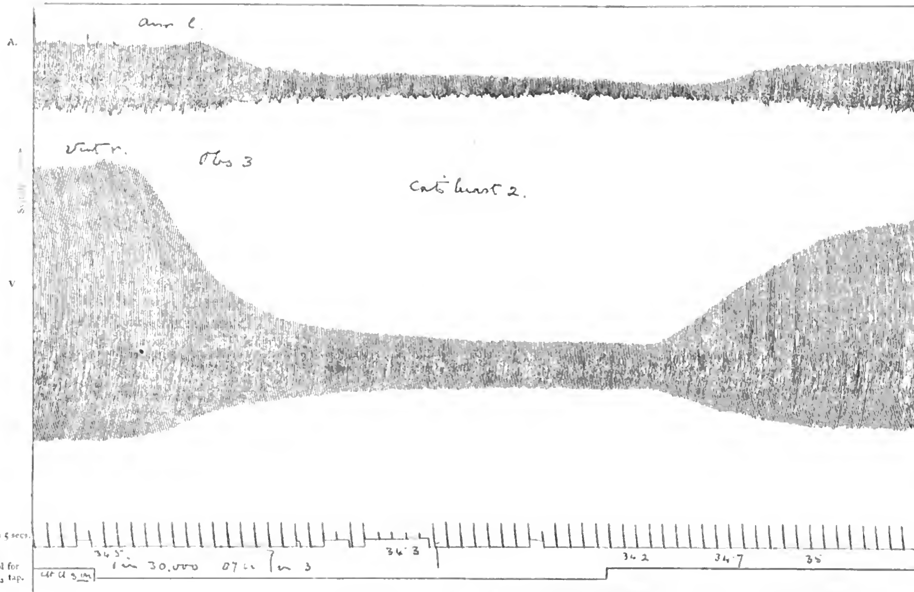


Fig. 8

VI.—Heart 21, Observation 3. Cat's Heart perfused with $CHCl_3$, 50 mg. per Litre (0·005 per cent.) of modified Ringer's solution for three minutes.

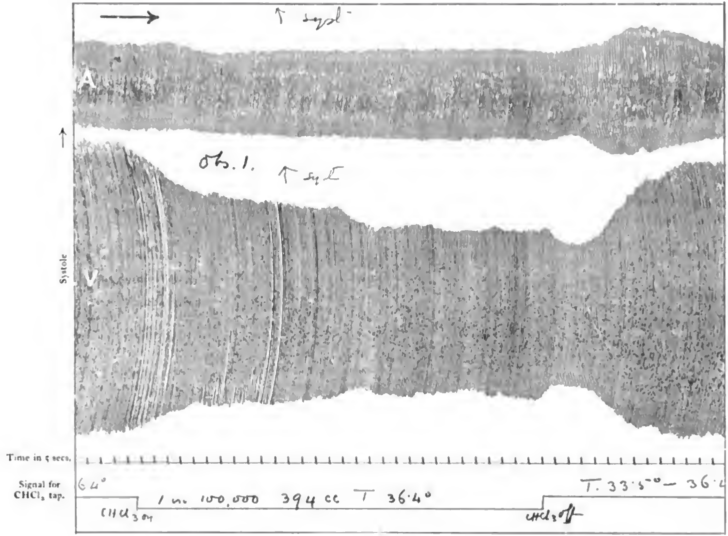


Fig. 9

X.—Heart 9, Observation 1. Cat's Heart; 15 mg. $CHCl_3$ per Litre of modified Ringer's solution.

The amplitude of the heart's beat has in these perfusions been as a fact somewhat less reduced than with corresponding dosage exhibited in 'RINGER.' The results are given in Table XIII.

INFLUENCE OF TEMPERATURE

We have been careful to note the temperature of the fluid entering the coronary system. We have performed control observations on the effect on the beating of the heart of gradual and of more sudden variations of temperature in the fluid supplied to the heart. As to the former, the exhaustive enquiries by NEWELL MARTIN (6 and 2) and by LANGENDORFF (7) stand. So definite a quickening of the cardiac rhythm follows *pari persen*, with gradual raising of its temperature, that NEWELL MARTIN formulated a numerical law correlating the two. LANGENDORFF in result of his own researches modifies NEWELL MARTIN's ratio.

Our observations on the effect of $CHCl_3$ solutions on the heart were conducted between 34° C. and 38° C. We find from our control experiments that the slight variations in temperature occurring in our individual $CHCl_3$ experiments do not confuse the issues raised and studied in those experiments.

It is the slight rapid changes that have required guarding against by us, namely, when one or other solution was first turned on. This source of error is avoided by the 3-way taps. The tap has been turned so as to discharge by the side path any fluid that has lain in the short tubing outside the water bath (though jacketed in cotton wool). Our aortic thermometer has shown us that by this means we have usually avoided even slight alterations of temperature. The readings of the thermometer have been always recorded. Such control experiments as we have made on this point show that the influence of the more sudden slight changes is of the same order as the considerably more gradual changes which we have also made observations upon, the effects of which were recorded by NEWELL MARTIN and LANGDENDORFF. In our fuller report we shall furnish further evidence to this effect.

EXCITATION OF THE CARDIAC NERVES

The cardiac nerves, lying in front of the root of the lung, were faradized, and their effect on the isolated heart recorded on many occasions. Typical vago-inhibitory effects on the auricle, and also on the ventricle, were almost uniformly obtained. The inhibitory effect was more marked on auricle than on ventricle. (Fig. 11). It was usually succeeded by very marked augmento-accelerator action, lasting many seconds, or even some minutes.

Chloroformization does not easily abolish these effects of stimulation. When the contractions were deeply reduced, almost to extinction, by the $CHCl_3$ perfusion, stimulation of the cardiac nerve would still cause slowing frequency-rate or diastolic standstill ; this was followed often by marked augmentation or acceleration, or both.

When the beat had been reduced by the $CHCl_3$ solution to extinction, to observe any inhibitory effect, of course, became impossible, but on faradizing the cardiac nerve striking augmento-accelerator effects were often observed. In some cases the heart, when it had been rendered flaccid and motionless by the $CHCl_3$ perfusion, was, by a brief faradization of the cardiac nerve, at once thrown into vigorous action, and remained so for a couple of minutes or so, in spite of continued administration of the $CHCl_3$ solution all the time. Fig. 12 illustrates this. $CHCl_3$ in ·002 per cent. had been already administered to the heart for ninety-five seconds, and the beat of the ventricle had entirely ceased from the first minute, when cardiac nerve was stimulated for fifteen seconds. In seven seconds from commencement of the stimulation the ventricle, although there was no remission of the $CHCl_3$ administration, recommenced beating. It soon attained an extraordinary force and frequency. It continued to beat for about 100 seconds, giving a series of 196 beats, in spite of the unremitted continued perfusion with the $CHCl_3$ solution.

Influence of Duration of Exhibition of the $CHCl_3$ Solution

A notable feature of the action of $CHCl_3$ in dilute solution on the isolated mammalian heart is absence of all cumulative aggravation. The solution produces its full effect in a comparatively few seconds, *e.g.*, seventy seconds after reaching the coronary system. Further prolongation of the administration does not render the depression more profound, but merely maintains it. On the discontinuance of the administration the effect passes off at once, in almost as few seconds as it took for its establishment.

With strong $CHCl_3$ solutions the duration of the administration does, no doubt, aggravate the depression of vitality. Thus with one per thousand $CHCl_3$ solution the heart that is not killed by perfusion for thirty seconds is killed by perfusion for sixty seconds. But these strong solutions do not come within the scope of therapeutic enquiry. Of moderate and weak solutions it may be confidently asserted that on the isolated mammalian heart their influence is marked by absence of cumulative effect. In some of our observations we have kept the chloroform solution, even in the strength of 150 mgrms. per litre of the Ringer's solution (*i.e.*, $CHCl_3$ in ·015 per cent. dilution) flowing continuously through the blood-vessels of the heart for twenty minutes at a time. Even over such periods as these no cumulative effect has been obvious; indeed, the depression of the heart's beat has been slightly less at the end than at the outset of that time (see following paragraph), and on replacing the chloroform solution by the pure Ringer's solution the recovery of the cardiac activity to its full previous degree has occurred absolutely promptly. For instance, in fifty seconds after replacement of the chloroform solution by the unchloroformed solution the heart has been beating as vigorously as it had been prior to the exhibition of the drug, and in spite of its forced abeyance for twenty minutes under the action of the drug.

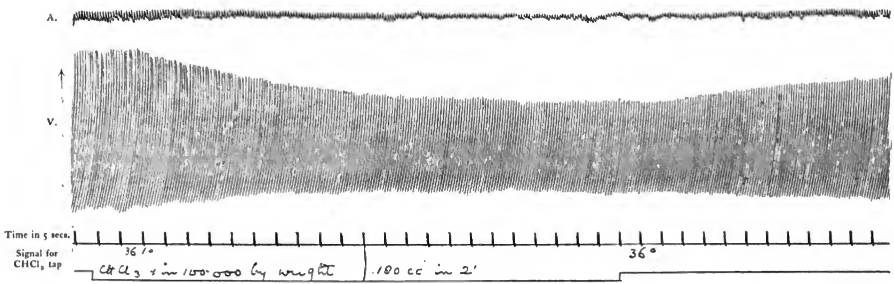


FIG. 10

XI.—Heart 39, Observation 4. Cat's Heart; $CHCl_3$ 10 mg. per Litre modified Ringer's solution (1 in 150,000 by volume) for two minutes. Pressure = 108 mm. Hg.

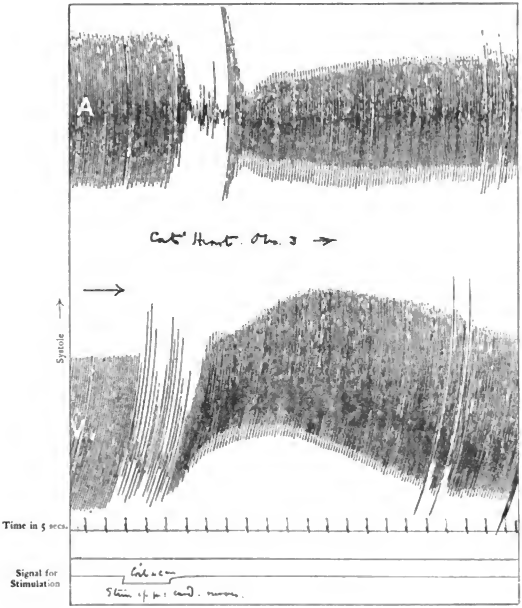


FIG. 11

Heart 31, Observation 3. Cat's Heart perfused with modified Ringer's solution; Precardiac Nerve stimulated for twelve seconds.

A second point of interest is the following. During the continuance of a prolonged administration to the heart of a weak or moderately strong $CHCl_3$ solution, the heart seems to become gradually less and less susceptible to the dose that is being employed. A kind of temporary and partial immunity seems to become established. This is exemplified by many of our tracings. We append pieces from two such tracings, Hearts 24 and 26 (Figs. 4 and 5). The apparent wearing off of the effect is not due to any real decrease in the strength of the solution. The same solution, that at end of a prolonged administration was not depressing the cardiac activity so potently as it had done at the outset of the observation, on being, after a brief pause, readmitted to the heart, immediately acted as potently as at outset once more. We have been accustomed to denote this for convenience in the laboratory as 'immunity;" but the relative unsusceptibility thus attained was very short lasting.

Excitatory Action of Chloroform

Especially with stronger solutions of the drug, it was usual for the first effect of the administration to be a distinct though slight increase in amplitude of both auricular and ventricular contractions. The fact that strong solutions left as an after effect on the ventricle a condition of inco-ordinate fibrillar convulsion likewise suggests an irritative excitatory effect of such doses of the drug.

After cessation of the administration of the drug in moderate dose it was not unusual for the recovery of the beat to pass over for a short time into a condition of super-activity. Many of our tracings illustrate this. We were at first inclined to attribute this to some slight asphyxia of the cardiac tissue. Control observations with boiled-out modified Ringer's solution, administered under nitrogen pressure, showed, however, that the after effect of the $CHCl_3$ solution cannot be clearly and simply explained by asphyxia. Moreover, our $CHCl_3$ solutions were as oxygenated and delivered under the same oxygen pressure as were our chloroform-free solutions. It may, however, be that under the action of the chloroform the tissue allows an accumulation of waste products that it excretes freely in its unchloroformed condition, and that these effete decomposition products make an excitatory effect which they can exert on the tissue patent after disappearance of the chloroform depression.

A similar condition might explain the gradual reduction in the depressant effect of a constantly-maintained strength of $CHCl_3$ solution continuously exhibited to the tissue over a period of several minutes duration, *e.g.*, five to twenty minutes. This reduction does, as stated and illustrated above, actually occur.

We have repeatedly noticed that the amount of perfused solution passing through the coronary circulation per unit time considerably diminishes soon after the $CHCl_3$ containing solution has replaced the $CHCl_3$ free solution. Since the pressure and temperature of the solutions supplied was the same in both cases, the diminution

of flow indicates a vasoconstrictor action by the $CHCl_3$ on the cardiac vessels; for the difference was so great as to be inexplicable by the loss of the power of the cardiac contractions or by a slight increase in the viscosity of the solution. [Subsequent to the printing of this paragraph of our *Report to the Committee* there has appeared a note by Professor SCHAFER and Dr. SCHARLIEB[2] on the action of chloroform on the blood-vessels, from which it is clear that chloroform causes contraction of blood-vessels through which it is perfused].

ALTERATION IN FREQUENCE OF THE BEAT-RATE

As several times mentioned above, we have remarked, with surprise, the usual absence of any alteration of frequence or rhythm of beat-rate in the isolated mammalian heart, perfused with dilute chloroform solution. Certainly, in most cases, the heart-beat in our experiments has suffered reduction in its amplitude in degree varying with the concentration of the chloroform, without suffering any appreciable change in beat-rate either as to frequence or to rhythm. But we have not yet measured out our curves sufficiently to say that diminution of frequence does never occur.

A.

V.

Time in 5 secs

Signal for chloroform

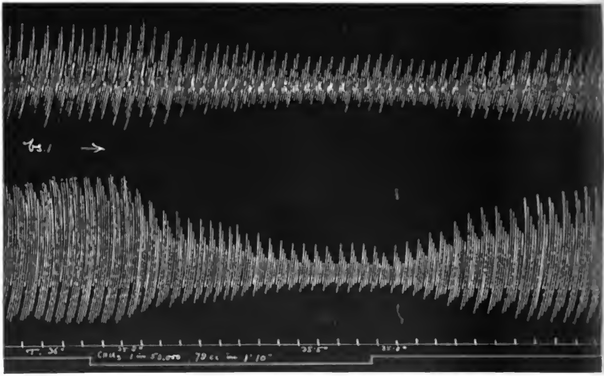


Fig. 13. Cat's heart. Experiment 27, observation 1. $CHCl_3$ one part in 50,000 of modified RINGER's fluid; 78 c.c. perfused in one minute ten seconds.

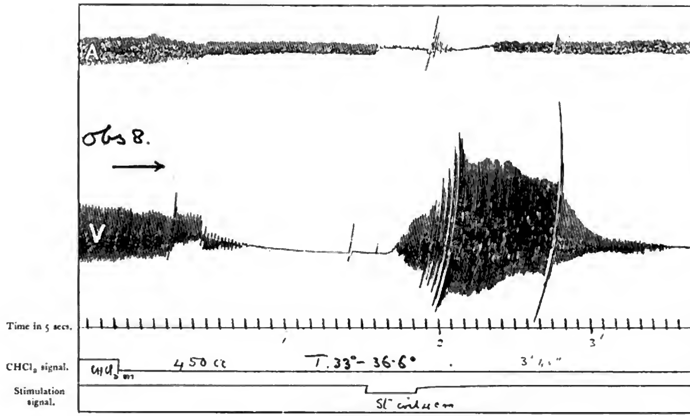


FIG. 12

VIII.—Heart 8, Observation 8. Resuscitation of heart by stimulation of Precardiac Nerve during $CHCl_3$ administration (1 in 50,000) in Ringer's solution.

How little, if at all, the cardiac rhythm is affected by $CHCl_3$ is shown well if the heart beats in groups. The amplitude of the individual beats is chiefly determined by the length of the preceding diastole.* If that be short the following beat is less ample, if long more ample. Under $CHCl_3$ the group-form persists practically unchanged (cf. Fig. 13). In some cases, however, $CHCl_3$ sets aside a pre-existing irregularity manifest as group beats.

The Dosage for the Heart

It is the concentration of the $CHCl_3$ in the perfused solution which seems to practically alone decide the depth of the depression of activity produced. Mere continuance of administration of fresh quantities of the drug does not, if the fresh quantities do not involve greater concentrations than those already employed, aggravate the paralysant action further. In our experiments such high strengths of chloroform as 150 mgrms. per litre diluting fluid (·015 per cent. $CHCl_3$) have perfused the whole coronary system uninterruptedly for twenty minutes at a time without, in all that time, producing the slightest aggravation of the cardiac depression established initially in the first ninety seconds. The gradation of the action of the drug seems therefore a function of its tension in the solvent. The results of our dosage experiments suggest that the cardiac tissue exposed to a fluid of a certain $CHCl_3$ pressure takes up almost at once a certain quantity, and that further continued exhibition of the drug under the same tension does not lead to further relative absorption of it by the tissue.

In recent years Dr. Waller has demonstrated, more especially by galvanometric records of the action-currents in isolated frog's nerve, the arithmetic grading of the depth of chloroform narcosis obtainable by definite grading of the admixtures of $CHCl_3$ with the air of the moist chamber containing the nerve. His presidential address to the Section of Physiology at the Montreal Meeting of the British Medical Association, 1897 (*Brit. Med. Journal*, November 20, 1897), was largely devoted to this theme. In it he pointed out the close analogy between frog's heart and frog's nerve in their reaction to various anaesthetics. Our results on $CHCl_3$ dosage in fluid supplied to the coronary circulation of the mammalian heart reveal just such a gradation as that found by Dr. Waller in nerve treated by chloroform vapour. The possibility of such grading of depression by dosage is one on which Dr. Waller[9] has often insisted as of practical importance. We are able to confirm it for the dosage of the isolated mammalian heart.

A form of expressing the $CHCl_3$ dosage, less instructive than that of giving its solution concentration, but nevertheless usefully showing its power upon the heart, is to give the weight of $CHCl_3$, which reduces the heart beat by a definite amount,

* Cf. R. S. Woodworth, *Amer. Jnl. of Physiology*, 1902

when offered to it at a certain solution (osmotic) pressure. Thus, in our experiments, the beat of Heart 8 was reduced by 92 per cent. by administration to it of 1·4 milligrammes of $CHCl_3$ offered to it as a ·003 per cent. solution (in RINGER's fluid), that is, under a solution pressure (assuming $CHCl_3$ to be a perfect monomolecular gas) of 4·79 mm. Hg., at temperature 37° C. Stated in amounts calculated per gramme of heart tissue (Heart 8 weighs 12·19 grammes), 0·114 milligrammes per gramme cardiac tissue offered under that pressure practically annulled the heart's action. In other words, about 300 cub. cent. of a 2 per cent. admixture of chloroform with air administered to an adult man, if its chloroform all reached the heart, and reached it under the above solution pressure, would, for the time being, practically annul cardiac action.

The above figure is given because it may serve to indicate the sensitiveness of the mammalian heart to chloroform. The practical problem requires many more data than we possess at present before it can be completely dealt with. Of chloroform inhaled, what portion enters the coronary blood-vessels? What tension of chloroform in air inhaled will, through the pulmonary membrane, charge the blood so as to give therein an osmotic pressure of 5·01 mm. Hg., as in a RINGER's fluid containing 30 mgrms. $CHCl_3$ per litre? From Mr. VERNON HARCOURT's data, furnished in last year's Report of the Committee* (Appendices, paper II, pp. 15, 16), chloroform in such a dilution in water at 37° C. would have a vapour tension of ·55 mm. Hg. In the modified RINGER's solution we have used for perfusion, it may be supposed that the tension would not be appreciably different from that in water. But the tension of chloroform or any dilution with blood is certainly less—probably far less—than for the same dilution with RINGER's fluid. The experiments of POHL, HARCOURT, and WELLS all indicate this. If the physiological activity of the chloroform is graded by its solution (osmotic) pressure, as we infer it is, observations easily made indicate that for the same dilution that pressure is much lower in blood than in RINGER's fluid. We find, for instance, tadpoles much more speedily paralysed by chloroform exhibited in RINGER's fluid than in blood.

Our observations were carried out as follows:—Into two similar shallow glass dishes, provided with glass covers, equal quantities of equal dilutions of $CHCl_3$ in RINGER and in defibrinated blood (pig), respectively, are placed—the $CHCl_3$ in 'RINGER' into one vessel, that in blood into the other. Two tadpoles are chosen similar in size and development. One (A) is placed in the one vessel, the other (B) in the other. Both A and B swim about freely, and gradually show signs of decreasing vigour and activity. The time taken for A and B respectively to become motionless and irresponsive when touched is noted. The chloroformed animal is, as soon as paralysed, at once removed to fresh water; the time is then noted which is required for it to regain its motility. Both animals having been allowed to recover perfectly in the fresh water, both are then again put into the chloroform containing fluids, but A now into the fluid previously occupied by B, and *vice versa*. The times of anaesthetization and subsequent recovery are then again observed for both A and B. The following are examples of such times:—

* *British Medical Journal*, July 12, 1902

TADPOLES

$CHCl_3$ 1·5 per thousand 'modified RINGER's solution,' and $CHCl_3$ 1·5 per thousand defibrinated blood, unlaked (pig). (1 in 1,000 by volume).

Chloroform in 'Ringer'		Chloroform in Blood	
Reflexes gone	Recovery	Reflexes gone	Recovery
50 secs.	8 mins.	4 mins. 50 secs.	5 mins.

$CHCl_3$ ·75 per thousand 'modified RINGER's fluid,' and $CHCl_3$ ·75 per thousand defibrinated blood, unlaked (pig). (1 in 2,000 by volume).

Chloroform in 'Ringer'			Chloroform in Blood		
	Reflexes gone	Recovery		Reflexes gone	Recovery
Tadpole A	2 mins. 30 secs.	2 mins. 25 secs. (begins)	Tadpole B	4 mins. (not fully narcotized)	revives at once
„ B	2 mins. 15 secs.	4—5 mins	„ A	7 mins. hardly narcotized	revives at once

$CHCl_3$ ·75 per mille 'modified RINGER's fluid,' compared with CHCl 1·5 per mille defibrinated blood, unlaked (pig).

Chloroform in 'Ringer'			Chloroform in Blood		
	Reflexes gone	Recovery		Reflexes gone	Recovery
Tadpole E	1 min. 40 secs.	3 mins. 25 secs.	Tadpole F	1 min. 25 secs.	3 mins.
„ F	1 „ 25 „	4 „ 15 „	„ E	1 „ 15 „	40 secs. (begins)
„ G	1 „ 50 „	2 „	„ H	2 „	1 min. „
„ H	2 „ 10 „	3 „	„ G	1 „ 55 „	30 secs. „

The solution of chloroform 1·5 per mille in blood was made by adding 20 c.c. of 5 per mille solution of $CHCl_3$ in 'modified RINGER,' to 80 c.c. of the fresh defibrinated blood.

It is thus seen that the physiological effect of chloroform in RINGER's fluid is much greater than that of the same percentage of chloroform in blood. As tested on tadpoles, $CHCl_3$ in ·75 per mille dilution in RINGER's fluid is as powerfully narcotic as twice that percentage offered, not in RINGER's fluid but in slightly diluted blood. Were the blood quite undiluted, the contrast would presumably be stronger still.

Mammalian Heart Perfused with Diluted Blood Containing 100 mgrms. of Chloroform per Litre

An experiment in which the heart was perfused with diluted blood showed the $CHCl_3$ to be much less potent in this medium than in 'modified Ringer's solution.' At a strength of 1 in 10,000 by weight or 100 mgrms. to a litre, the ventricle's beat was reduced by 30 per cent. in *Observation* 1, by 32 per cent. in *Observation* 2. The reduction which we should expect a similar dilution of chloroform in modified Ringer's fluid to produce, would be between 80 per cent. and 90 per cent. of the ventricular beat. The amounts perfused were: *Observation* 1, 9·5 c.c. in one minute fifty seconds, *Observation* 2, 10 c.c. in three minutes twelve seconds. The blood was diluted with an equal volume of Locke's solution.

Observations on the Human Heart

In two instances we have had opportuity to examine $CHCl_3$ dosage for the isolated human heart. In the first case, observations were commenced six hours *post-mortem*; in the second, a still-born child, three hours after delivery. The fluid perfused was, in the former case, 'modified Ringer,' in the latter, 'Locke's solution.' With a strength of $CHCl_3$ solution of 100 mgrms. per litre (1 in 10,000), the reduction (average) of the auricular beat was 76·5 per cent.; of the ventricular was 92 per cent. The results were therefore quite in conformity with those given by the cat hearts. Fig 14 shows one of the records obtained in the second of the above cases.

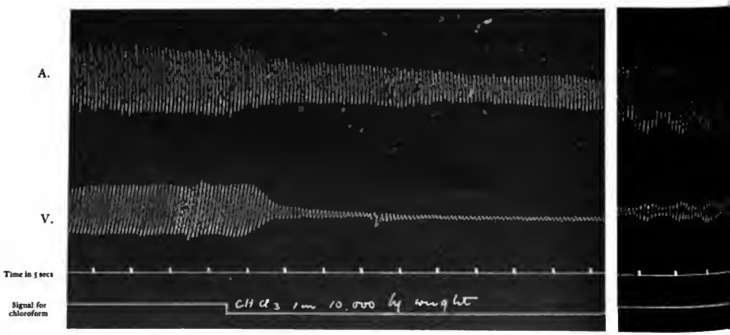


Fig. 14. Experiment 52. Observation 3. Human heart perfused with $CHCl_3$ 100 mgrms. per litre, Locke's solution. Time marked every fifth second. An interval of two minutes between the end of Trace 1 and beginning of Trace 2. $CHCl_3$ was perfused for eighty seconds. A, auricular beat; V, ventricular beat.

Conclusion

The conclusion seems unavoidable that the heart muscle rapidly takes up $CHCl_3$ offered to it in the vessels of its coronary system, and that the quantities it takes increase with increasing tension of the chloroform in the solution circulating through it.

The osmotic pressure of $CHCl_3$ in a dilution of 1 in 100,000 of physiological saline is about 1·61 mm. Hg., at 37° C. This is the lowest chloroform tension we have worked with. With very strong solutions, such as 1 per 1,000, toxic after effects complicate the simpler relationship which holds for moderate and weak solutions.

Equilibrium is rapidly (*e.g.*, in fifty seconds) established between the intramuscular chloroform and the circulating or perfused chloroform. When that is established, further exhibition of the circulating chloroform leads to no further increase of chloroform paralysis of the tissue, provided the solution-tension of the circulating chloroform is not altered. Indeed, if that solution-tension is maintained, but not increased, there gradually ensues in the course of minutes (*e.g.*, ten to twenty minutes) a distinct diminution of the cardiac depression. Should, however, the solution-tension be altered, alteration in the depth of chloroform paralysis ensues in the sense that if the tension of $CHCl_3$ is increased, corresponding aggravation of the paralysis of the tissue immediately follows, while if the tension of the circulating $CHCl_3$ be lessened, the paralysis of the tissue is immediately correspondingly diminished. The speed and completeness with which this recovery from functional depression follows forthwith upon replacement of the chloroform containing fluid by fluid free from chloroform is a remarkable and characteristic feature of the action of chloroform on the heart in weak and moderate concentrations. It suggests that, in obedience to the direction of the gradient of osmotic pressures, the chloroform in the muscle passes back from the muscle into the fresh fluid supplied at zero chloroform pressure. In this respect the chloroform behaves as though it were in solution in the muscle. Can this drug poison the muscle without entering into chemical combination with its substance? Does it act as an anti-katalysator on the ferment processes which lie at root of the normal functional activity of the contracting tissue of the heart?

As to the question of differences of susceptibility to chloroform of different individual hearts, our observations indicate that such differences do occur in cats' hearts. The differences have been especially obvious to us in our experiments with weak solutions. The degrees of the difference can be studied by references to the table of data appended to the report.

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Report of the Special Chloroform Committee. Appendices. Paper II.

FIGURES

All the figures read from left to right. Where numerals are written next under the time record they indicate the temperature in centigrade scale recorded by the aortic thermometer at that time. The explanation of other parts of the tracings is given either on the tracings or in the accompanying text.

I. 1,500 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
($CHCl_3$ in ·15 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat	V. beat	Recovery
Heart 32 :—					
Obs. 3 ...	30 c.c.	30 secs.	Stopped in 19 secs. (after 25 beats)	Stopped in 15 secs. (after 21 beats)	Full. A. begins to beat in 75 secs.; V. a few secs. later.
Obs. 4 ...	113 c.c.	60 secs.	Stopped within 20 secs.	Stopped finally	Full in the case of A., beats commencing in 75 secs.; V. shows only fibrillar contractions
Obs. 5 ...	71 c.c.	62 secs.	do.	...	Full in A.
Obs. 6 ...	190 c.c.	2 mins.	do.	...	Fair in A.
Obs. 7 ...	240 c.c.	3 mins.	do.	...	do.
Obs. 8 ...	?	5 mins.	do.	...	do.

II. 750 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
($CHCl_3$ *in* ·075 *per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat	V. beat	Recovery
Heart 22 :—					
Obs. 1 ...	56 c.c.	50 secs.	Stopped	Stopped	Full. A. begins in 30 secs. ; V. begins in 1 min. 40 secs.
Obs. 2 ...	78 c.c.	1 min. 5 secs.	do.	do.	Full. A. begins within 35 secs. ; V. begins within 1 min. 40 secs.
Obs. 3 ...	308 c.c.	3 mins. 20 secs.	do.	do.	Full. A. begins in 1 min. 25 secs. ; V. begins about 1 min.
Obs. 4 ...	555 c.c.	5 mins. 5 secs.	do.	Stopped finally	Full, in A. begins in 2 mins. 15 secs.
Heart 24 :—					
Obs. 4 ...	40 c.c.	1 min.	Stopped within 30 secs.	Stopped within 30 secs. finally	Full in A. ; V. shows fibrillar contractions only
Obs. 5 ...	190 c.c.	3 mins. 15 secs.	do.	...	Almost full in A., fibrillar contractions in V.
Obs. 6 ...	940 c.c.	20 mins.	do.	...	As before
Heart 25 :—					
Obs. 1 ...	60 c.c.	1 min.	Stopped	Stopped	Full
Obs. 2 ...	70 c.c.	1 min.	do.	do.	do.
Obs. 3 ...	?	57 secs.	do.	do.	do.
Obs. 4 ...	75 c.c.	1 min. 5 secs.	do.	do.	do.
Obs. 5 ...	280 c.c.	3 mins.	do.	do.	do.
Obs. 6 ...	440 c.c.	5 mins. to 6 mins.	do.	do.	do.
Obs 7 ...	470 c.c.	About 9 mins.	do.	Stopped finally	Slight, after 15 mins.

III. 300 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
($CHCl_3$ *in* ·03 *per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat	V. beat	Recovery
Heart 23 :—					
Obs. 1 ...	52 c.c.	1 min. 8 secs.	Stopped within 30 secs.	Stopped within 50 secs.	Full. V. in 3 mins.
Obs. 2 ...	155 c.c.	3 mins.	As before	As before	Full. V. in 4 mins.
Obs. 3 ...	260 c.c.	5 mins.	do.	do.	Full. V. in 2 mins. 25 secs.
Obs. 4 ...	660 c.c.	10 mins.	do.	do.	Full. V. in 1 min. 15 secs., beat less regular
Obs. 5 ...	300 c.c.	5 mins.	Stopped within 25 secs.	Stopped in 85 secs.	Full. V. in 1 min. 35 secs.
Obs. 6 ...	1,775 c.c.	18 mins.	Stopped	Stopped	Full. V. in 40 secs.
Heart 26 :—					
Obs. 1 ...	97 c.c.	57 secs.	Almost stopped	Practically stopped	Full
Obs. 2 ...	280 c.c.	3 mins.	Practically stopped	do.	Full. V. in 60 secs.
Obs. 3 ...	?	16 mins.	Stopped	Stopped	Full ultimately

IV. 150 MGRMS. $CHCl_3$ PER LITRE 'MODIFIED RINGER'S' FLUID
(*$CHCl_3$ ·015 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 1 :—					
Obs. 1 ...	?	63 secs.	83 per cent.	Practically stopped	Full
Obs. 2 ...	?	1 min. 45 secs.	83 per cent.	do.	do.
Obs. 3 ...	156 c.c.	2 mins. 22 secs.	86 per cent.	Stopped	do.
Heart 24 :—					
Obs. 1 ...	113 c.c.	3 mins.	Stopped	98 per cent.	Full. A. begins in about 30 secs.
Obs. 2 ...	200 c.c.	5 mins.	do.	99 per cent.	As before
Obs. 3 ...	770 c.c.	20 mins.	do.	Practically stopped, but regains 10 per cent. of its initial value	do.
Heart 26 :—					
Obs. 4 ...	230 c.c.	3 mins.	Stopped	Stopped	Full
Obs. 5 ...	800 c.c.	10 mins. 15 secs.	Practically stopped, but a small beat returns before $CHCl_3$ solution is turned off	Practically stopped, but regains 17 per cent. of its value before $CHCl_3$ solution is turned off	do.
Obs. 6 ...	380 c.c.	7 mins.	Practically stopped	Practically stopped, but is beating again before $CHCl_3$ solution is off	do.
Heart 29 :—					
Obs. 5 ...	160 c.c.	3 mins. 5 secs.	Stopped	Stopped	Full
Obs. 6 ...	320 c.c.	5 mins. 5 secs.	do.	do.	do.

IV—*Continued*

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 35 :—					
Obs. 1 ...	?	5 mins. 10 sec.	92 per cent.	89 per cent.	Full
Obs. 2 ...		2 mins. 35 secs.	Practically stopped	88·5 per cent.	do.
Obs. 3 ...		2 mins. 10 secs.	do.	87 per cent.	do.
Obs. 4 ...		45 secs.	do.	Practically stopped	do.
Obs. 5 ...		1 min. 35 secs.	do.	do.	Very imperfect, with fibrillar contractions
Heart 38 :—					
Obs. 1 ...	?	42 secs.	Practically stopped	Practically stopped	Full
Obs. 2 ...		1 min. 45 secs.	do.	do.	do.
Obs. 3 ...		1 min. 50 secs.	do.	do.	do.
Obs. 4 ...		1 min. 38 secs.	do.	do.	do.
Obs. 5 ...		43 secs.	Nearly stopped	Nearly stopped	do.

Average reduction of A. 96·9 ; of V. 97·6 per cent.

V. 75 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·0075 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 2 :—					
Obs. 1 ...	1,800 c.c.	22 mins. 40 secs.	56 per cent. $>$ 34 per cent. $<$ 64 per cent.	80 per cent. $>$ 67 per cent.	Full
Heart 16 :—					
Obs. 1 ...	200 c.c.	3 mins. 9 secs.	87 per cent.	85 per cent.	do.
Obs. 2 ...	280 c.c.	4 mins. 18 secs.	Practically stopped	90 per cent.	do.
Obs. 3 ...	240 c.c.	3 mins. 9 secs.	do.	Practically stopped	do.
Heart 18 :—					
Obs. 1 ...	166 c.c.	3 mins. 5 secs.	88 per cent.	Not recorded	do.
Heart 31 :—					
Obs. 1 ...	190 c.c.	3 mins.	85 per cent. $>$ 66 per cent.	97 per cent. $>$	do.
Obs. 2 ...	160 c.c.	3 mins. 3 secs.	70 per cent. $>$ 62 per cent.	80 per cent. $>$ 76 per cent.	do.

Average reduction, Heart 16, 3 obs. : A., 95·7 per cent. ; V., 91·7 per cent.
" " " 31, 2 obs. ; A., 77·5 per cent. ; V., 88·5 per cent.
Mean reduction in three hearts : A., 79 per cent. ; V., 86·7 per cent.

VI. 50 mgrms. $CHCl_3$ per Litre of Modified Ringer's Solution

(*$CHCl_3$ in ·005 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 5 :—					
Obs. 2 ...	?	40 secs.	50 per cent.	Stopped	Full
Obs. 3 ...	110 c.c.	2 mins. 32 secs.	73 per cent.	do.	do.
Obs. 4 ...	52 c.c.	1 min. 15 secs.	70 per cent.	do.	do.
Obs. 5 ...	168 c.c.	3 mins. 32 secs.	88 per cent.	do.	do.
Obs. 6 ...	100 c.c.	1 min. 57 secs.	75 per cent.	do.	do.
Heart 6 :—					
Obs. 1 ...	105 c.c.	3 mins.	75 per cent.	92 per cent.	...
Heart 20 :—					
Obs. 2 ...	163 c.c.	2 mins. 55 secs.	29 per cent.	39 per cent.	Full
Heart 21 :—					
Obs. 1 ...	167 c.c.	3 mins.	64 per cent.	47 per cent.	Full
Obs. 2 ...	97 c.c.	3 mins. 5 secs.	61 per cent.	82 per cent.	do.
Obs. 3 ...	87 c.c.	3 mins.	75 per cent.	83 per cent.	do.
Obs. 4 ...	81 c.c.	3 mins.	69 per cent.	84 per cent.	do.
Obs. 5 ...	90 c.c.	3 mins.	87 per cent.	85 per cent.	do.
Obs. 6 ...	93 c.c.	3 mins. 4 secs.	89 per cent.	77 per cent.	do.

Average reduction, Heart 5, 5 obs. : A., 71·2 per cent. ; V., 100 per cent.
" " " 21, 6 obs, : A., 74 per cent. ; V., 76·3 per cent.
Mean reduction in four hearts : A., 62·3 per cent. ; V., 76·8 per cent.

VII. 38 mgrms. $CHCl_3$ per Litre of Modified Ringer's Solution
($CHCl_3$ in ·0038 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 7 :—					
Obs. 1 ...	103 c.c.	1 min. 35 secs.	30 per cent.	Practically stopped	Full
Obs. 2 ...	20 c.c.	27 secs.	30 per cent.	80 per cent.	do.
Obs. 3	127 c.c.	2 mins. 18 secs.	75 per cent.	Stopped	do.
Obs. 4 ...	125 c.c.	2 mins. 13 secs.	70 per cent.	do.	do.
Obs. 5 ...	440 c.c.	9 mins. 50 secs.	69 per cent.	Stopped, but begins again before $CHCl_3$ solution is off	do.
Heart 19 (irregular beats) :—					
Obs. 1 ...	180 c.c.	2 mins. 53 secs.	Over 70 per cent.	Over 70 per cent.	do.
Obs. 2 ...	184 c.c.	3 mins. 5 secs.	do.	do.	do.
Obs. 3 ...	173 c.c.	3 mins.	do.	do.	do.

Average reduction, Heart 7, 5 obs.: A., 55 per cent.; V., 96 per cent.

VIII. 30 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·003 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 8 :—					
Obs. 1 ...	92 c.c.	2 mins. 7 secs.	63 per cent.	95 per cent.	Full
Obs. 2 ...	111 c.c.	2 mins. 35 secs.	82 per cent.	Stopped	do.
Obs. 3 ...	200 c.c.	3 mins. 40 secs.	70 per cent.	do.	do.
Obs. 4 ...	185 c.c.	3 mins. 5 secs.	68 per cent.	Practically stopped	do.
Obs. 5 ...	75 c.c.	87 secs.	60 per cent.	92 per cent.	do.
Obs. 6 ...	55 c.c.	45 secs.	65 per cent.	94 per cent.	do.
Obs. 7 ...	210 c.c.	195 secs.	66 per cent.	95 per cent.	do.
Obs. 8 ...	450 c.c.	9 mins. 17 secs.	62 per cent.	Stopped	do.
Heart 27 :—					
Obs. 1 ...	78 c.c.	1 min. 10 secs.	53 per cent.	69 per cent.	do.
Obs. 2 ...	150 c.c.	3 mins.	50 per cent.	83 per cent.	do.
Obs. 3 ...	140 c.c.	3 mins.	69 per cent.	89 per cent.	do.
Obs. 4 ...	140 c.c.	3 mins.	57 per cent.	82 per cent.	do.
Heart 28 :—					
Obs. 1 ...	135 c.c.	3 mins.	28 per cent.	25 per cent. >	do.
Obs. 2 ...	120 c.c.	3 mins.	Not recorded	36 per cent. >	do.
Obs. 3 ...	170 c.c.	5 mins.	56 per cent.	25 per cent. >	do.
Obs. 4 ...	155 c.c.	4 mins.	57 per cent.	40 per cent. >	do.

VIII—*Continued*

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 28 :— (*continued*) Obs. 5 ...	550 c.c.	10 mins.	62 per cent. >	40 per cent. >	Full
Obs. 6 ...	380 c.c.	5 mins. 5 secs.	62 per cent. >	36 per cent.	do.
Heart 29 :— Obs. 4 ...	120 c.c.	3 mins.	45 per cent.	15 per cent.	do.
Heart 30 :— Obs. 4 ...	90 c.c.	1 min. 12 secs.	86 per cent.	Not recorded	do.
Obs. 5 ...	240 c.c.	3 mins. 3 secs.	88 per cent.	do.	do.
Obs. 6 ...	51 c.c.	1 min. 7 secs.	83 per cent.	do.	do.

Average reduction, Heart 8, 8 obs. : A., 67 per cent. ; V., 97 per cent.
" " " 27, 4 obs. : A., 55·2 per cent. ; V., 80·7 per cent.
" " " 28, 6 obs. : A., 53 per cent. ; V., 22·6 per cent.
" " " 30, 3 obs. : A., 85·6 per cent.
" " " 29, 1 obs. : A., 45 per cent. ; V., 15 per cent.

Mean reduction : A. in five hearts = 61·2 per cent.
" " V. in four hearts = 53 per cent.

IX. 20 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·002 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. (left) beat reduced by	V. beat reduced by	Recovery
Heart 41 :—					
Obs. 1 ...	76 c.c.	2 mins.	48 per cent.	28 per cent.	Full
Obs. 2 ...	78 c.c.	1 min. 58 secs.	34 per cent.	15 per cent.	do.
Obs. 3 ...	140 c.c.	3 mins.	34 per cent.	50 per cent.	do.
Obs. 4 ...	260 c.c.	5 mins.	40 per cent.	38 per cent.	do.
Obs. 5 ...	68 c.c.	1 min. 30 secs.	?	28 per cent.	do.
Obs. 6 ...	124 c.c.	1 min. 55 secs.	A. right 35 per cent.	34 per cent.	do.
Obs. 7 ...	240 c.c.	3 mins 25 secs.	27 per cent.	35 per cent.	do.

Average reduction in A., 6 obs. = 36·33 per cent. ; V., 7 obs. = 32·57 per cent.

X. 19 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION
(*$CHCl_3$ in ·0019 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 14 :—					
Dog's, Obs. 1	1,700 c.c.	9 mins. 35 secs.	64 per cent.	Not recorded	Good
Obs. 3	1,800 c.c.	9 mins. 35 secs.	75 per cent.	...	do.

Average reduction = 69·5 per cent.

XI. 15 mgrms. $CHCl_3$ per Litre of Modified Ringer's Solution
($CHCl_3$ in ·0015 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 9 :—					
Obs. 1 ...	394 c.c.	2 mins. 30 secs.	10 per cent.	40 per cent.	Full
Obs. 2 ...	640 c.c.	4 mins. 40 secs.	50 per cent.	49 per cent.	do.
Obs. 3 ...	200 c.c.	1 min. 17 secs.	43 per cent.	37 per cent.	do.
Heart 15 :—					
Obs. 1 ...	810 c.c.	11 mins. 12 secs.	...	...	...
Obs. 5 ...	510 c.c.	6 mins. 55 secs.	20 per cent.	40 per cent. > 20 per cent.	Full
Heart 30 :—					
Obs. 1 ...	320 c.c.	3 mins. 5 secs.	18 per cent.	26 per cent.	do.
Obs. 2 ...	80 c.c.	1 min. 10 secs.	34 per cent.	Not recorded	do.
Obs. 3 ...	92 c.c.	1 min. 15 secs.	39 per cent.	do.	do.

Average reduction, Heart 9, 3 obs. : A., 34·3 per cent. ; V., 42 per cent.
" " " 30, 3 obs. : A., 30·3 per cent.
" " " 30, 1 obs. : V., 26 per cent.
Mean reduction in three hearts : A., 28·2 per cent. ; V., 38·4 per cent.

XII. 10 MGRMS. $CHCl_3$ PER LITRE OF MODIFIED RINGER'S SOLUTION

($CHCl_3$ in ·001 per cent. dilution ; or 1 in 150,000 by vol.)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 39 :—					
Obs. 1 ...	190 c.c.	1 min. 55 secs.	40 per cent.	11 per cent.	Full
Obs. 2 ...	170 c.c.	1 min. 47 secs.	45·5 per cent.	17 per cent.	do.
Obs. 3 ...	265 c.c.	3 mins.	47 per cent.	17 per cent.	do.
Obs. 4 ...	180 c.c.	2 mins.	44·5 per cent.	23 per cent.	do.
Obs. 5 ...	365 c.c.	3 mins. 55 secs.	27 per cent.	28·8 per cent.	do.
Heart 40 :—					
Obs. 1 ...	100 c.c.	2 mins.	77 per cent.	29 per cent.	do.
Obs. 2 ...	115 c.c.	2 mins. 3 secs.	45 per cent.	24 per cent.	do.
Obs. 3 ...	100 c.c.	2 mins.	58 per cent.	24 per cent.	do.
Obs. 4 ...	180 c.c.	3 mins.	57 per cent.	23 per cent.	do.
Obs. 5 ...	230 c.c.	3 mins. 10 secs.	40 per cent.	20 per cent.	do.

Average reduction in 10 obs. : A., 48·1 per cent. ; V., 21·67 per cent.

PERFUSIONS WITH LOCKE'S FLUID

XIII. 100 MGRMS. $CHCl_3$ PER LITRE OF LOCKE'S FLUID
($CHCl_3$ in ·01 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 55 :— Obs. 1 ...	?	1 min. 25 secs.	90 per cent.	96 per cent.	Full
Obs. 2 ...	45 c.c.	1 min. 13 secs.	88 per cent.	93 per cent.	do.
Obs. 3 ...	95 c.c.	2 mins. 3 secs.	96 per cent.	98 per cent.	do.
Heart 56 :— Obs. 1 ...	358 c.c.	13 mins. 15 secs.	Not recorded	95 per cent.—90 per cent.	Full
Obs. 2 ...	134 c.c.	4 mins. 55 secs.	do.	99 per cent.	do.
Obs. 3 ...	440 c.c.	15 mins. 30 secs.	do.	98 per cent.—76 per cent.	do.

Average reduction, Heart 55, 3 obs. : A., 91·3 per cent. ; V., 95·6 per cent.
" " Heart 56, 3 obs. : V., 97·3 per cent.
Mean reduction, V. in two hearts : 96·45 per cent.

XIV. 66 MGRMS. $CHCl_3$ PER LITRE OF LOCKE'S FLUID
($CHCl_3$ in ·0066 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 44 :— Obs. 2 ...	80 c.c.	1 min. 5 secs.	26 per cent.	40 per cent.	Full
Obs. 3 ...	250 c.c.	4 mins. 50 secs.	67 per cent.	75·5 per cent.	do.
Obs. 5 ...	100 c.c.	2 mins. 35 secs.	75 per cent.	63 per cent.	do.
Obs. 6 ...	62 c.c.	1 min. 45 secs.	87 per cent.	72·5 per cent.	do.
Obs. 7 ...	385 c.c.	12 mins. 20 secs.	85 per cent.	67·5 per cent. > 45 per cent.	do.

Average reduction, A. beat by 68 per cent. ; V. beat by 65·7 per cent.

XV. 50 MGRMS. $CHCl_3$ PER LITRE OF LOCKE'S FLUID
(*$CHCl_3$ in ·005 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 5 :— Obs. 1 ...	?	1 mins. 22 secs.	50 per cent.	39 per cent.	Full
Obs. 2 ...	?	2 min. 35 secs.	70 per cent.	42 per cent.	do.
Obs. 4 ...	65 c.c.	1 mins. 45 secs.	80 per cent.	63 per cent.	do.
Obs. 6 ...	310 c.c.	17 mins.	?	52 per cent. > 40 per cent.	do.

Average reduction, A. beat, 3 observations, 66·6 per cent.
" " V. beat, 4 observations, 49 per cent.

XVI. 38 MGRMS. $CHCl_3$ PER LITRE OF LOCKE'S FLUID
(*$CHCl_3$ in ·0038 per cent. dilution*)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 53 :— Obs. 1 ...	?	1 min.	42 per cent.	44 per cent.	Full
Obs. 2 ...	80 c.c.	2 mins. 15 secs.	42 per cent.	44 per cent.	do.
Obs. 3 ...	161 c.c.	5 mins. 10 secs.	45 per cent.	45 per cent.	do.
Obs. 4 ...	?	1 min. 40 secs.	52 per cent.	45 per cent.	do.
Heart 54 :— Obs. 1 ...	41 c.c.	1 min. 30 secs.	75 per cent.	56 per cent.	Full
Obs. 2 ...	50 c.c.	1 min. 58 secs.	78 per cent.	59 per cent.	do.
Obs. 3 ...	89 c.c.	?	79 per cent.	48 per cent.	do.
Obs. 5 ...	90 c.c.	3 mins.	50 per cent.	45 per cent.	do.
Obs. 6 ...	73 c.c.	3 mins.	52 per cent.	45 per cent.	do.
Obs. 7 ...	115 c.c.	5 mins.	47 per cent.	40 per cent.	do.

Average reduction, Heart 53, 4 obs. : A., 45·25 per cent. ; V., 44·5 per cent.
" " Heart 54, 6 obs. : A., 63·5 per cent. ; V., 48·83 per cent.
Mean reduction in two hearts : A., 54·7 per cent. ; V., 46·66 per cent.

XVII. 33 mgrms. $CHCl_3$ per Litre of Locke's Fluid
($CHCl_3$ in ·0033 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 46 :— Obs. 1 ...	75 c.c.	1 mins. 32 secs.	31 per cent.	34 per cent.	Full
Obs. 2 ...	86 c.c.	2 mins. 20 secs.	65 per cent.	57 per cent.	do.
Obs. 3 ...	150 c.c.	4 mins. 38 secs.	71 per cent.	61 per cent.	do.
Obs. 4 ...	70 c.c.	2 mins. 55 secs.	80 per cent.	63 per cent.	do.
Obs. 5 ...	84 c.c.	3 mins. 15 secs.	75 per cent.	56 per cent.	do.
Obs. 6 ...	365 c.c.	13 mins. 10 secs.	67 per cent. > 33 per cent.	63 per cent. > 34 per cent.	do.

Average reduction, A. beat by 64·83 per cent. ; V. beat by 55·66 per cent.

XVIII. 30 mgrms. $CHCl_3$ per Litre of Locke's Fluid
($CHCl_3$ in ·003 per cent. dilution)

No. of Heart	Quantity of $CHCl_3$ solution perfused	Duration of flow	A. beat reduced by	V. beat reduced by	Recovery
Heart 50 :— Obs. 1 ...	92 c.c.	1 min. 33 secs.	Not recorded	30 per cent.	Full
Obs. 2 ...	?	1 min. 57 secs.	do.	35 per cent.	do.
Obs. 3 ...	145 c.c.	2 mins. 55 secs.	do.	58 per cent.	do.
Heart 51 :— Obs. 1 ...	47 c.c.	2 mins.	38 per cent.	24 per cent.	do.
Obs. 2 ...	29 c.c.	1 min. 20 secs.	59 per cent.	49 per cent.	do.
Obs. 3 ...	50 c.c.	2 mins.	58 per cent.	26 per cent.	do.

Average reduction, Heart 50 : V. beat, 3 observations, 41 per cent.
" " Heart 51 : 3 observations, A. beat 51·6 per cent. ; V. beat 33 per cent.
Mean reduction in two hearts, V. beat 37 per cent.

EXPERIMENTS ON THE DETECTION OF B. TYPHOSUS IN INFECTED MATERIAL

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EXPERIMENTS ON THE DETECTION OF B. TYPHOSUS IN INFECTED MATERIAL

By EDWARD H. HUME, B.A., M.D.
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ALTHOUGH much has been done towards the detection of *B. typhosus* in contaminated material, by methods which utilize the differences in its chemical activity and in the morphology of its colonies from that of other organisms, very few investigators have laid much stress on its greater motility as compared with that of *B. coli*, etc., as a characteristic by which it might be recognized and isolated.

STODDART's[10] method is based on this characteristic, while that of HISS[6] utilizes all three features, its chemical activity, its morphology, and its motility.

GABRITSCHEWSKY[3], however, was the first to devise a method based solely on the motility of *B. typhosus*, and so far as the writer can determine, no other worker has suggested further adaptations of this principle.*

In connexion with other experiments on typhoid infection, repeated efforts were made by the writer to devise a simple form of GABRITSCHEWSKY's tube[3], such as could be used in the routine examination of faeces or even of water. Although it was proved possible, using a tube of this sort, to isolate *B. typhosus* in pure culture from artificial mixtures, and in one special instance, to isolate a motile bacillus from the stools and urine of a patient suffering from what is now called 'Para-typhoid' infection, yet the results were inconstant. Too much depended on the tightness with which the cotton-wool plug was inserted into the proximal end, and on the facility with which one could fill the tubes with the nutrient medium. Further study along this line was therefore discontinued for a time, only to be renewed after the publication of VON DRIGALSKI's and CONRADI's[1] results in the February of the present year (1902).

It was suggested by these writers that emulsions of faeces from suspected cases of typhoid fever, or other fluids suspected of containing *B. typhosus*, should be centrifugalized, and cultures made from the surface of the centrifugalized fluid at intervals of fifteen minutes for some hours. They report having been successful in thus isolating *B. typhosus* in many instances.

It seemed desirable to determine whether such centrifugalization really lessened the number of bacilli at the surface in any marked degree, thus bringing the greater motility of *B. typhosus* into play. Artificial mixtures of *B. typhosus* and *B. coli* were

* Hill[6] reports a series of experiments to determine the relation between the motility of bacteria and their ability to penetrate wet cotton, and concludes that their rate of passage varies with the relative activity of their motility. He does not, however, seem to have applied the principle in the isolation of pathogenic bacteria from infected material.

accordingly centrifugalized in an electric centrifuge (two thousand revolutions per minute) for lengths of time varying from fifteen to sixty minutes. Plates were made from the surface in the method outlined by VON DRIGALSKI and CONRADI,[1] and, although the number of bacilli was found to be less directly after the centrifugalization than just before, yet the difference was not striking.

It was evidently desirable to deliver the suspected material at the *bottom* of a tube, if possible, and to liberate it there into a large quantity of medium in such a way as to prevent bacilli from rising to the surface at once, as they would if the inoculation were directly into bouillon. The technique of the first experiment was suggested during the reading of an article by HARRIS,[4] 'Concerning an Improved Method of making Collodium Sacs.'

Experiment 1. *Gelatin capsules*

Into 10 c.c. of bouillon were inoculated one loop of a young bouillon culture of *B. typhosus*, and three loops of a similar culture of *B. coli*. Gelatin capsules containing 0·5 c.c. of the above mixture were deposited at the bottom of each of twelve sterile tubes, and over each capsule were poured 5 c.c. of 12 per cent. gelatin, and this was allowed to harden. The capsule was now completely imbedded in gelatin. After a few trials it was found desirable to place the tubes in the thermostat for fifteen minutes before allowing the gelatin to harden, in order to expel the air-bubble, which was of course present at the upper part of the gelatin capsule. Over the hardened gelatin there was poured, in six of the tubes, 15 c.c. of gelatin (12 per cent.), containing bile-salt (0·5 per cent.), and in the other six 25 c.c. of the same fluid.* There was now present at the bottom of each tube a mixture of typhoid and colon bacilli imbedded in gelatin, and above it a column of sterile gelatin, all of which was sure to melt as soon as placed in the thermostat. It was hoped by this method to give the typhoid bacilli the best possible chance to get away from the colon bacilli. Plates were made from the surface of each tube, the gelatin was allowed to harden, and then all twelve tubes were placed in the thermostat at 37° C. At intervals of one hour, the gelatin being again in a liquid state at that temperature, one loop was taken from the surface of each tube, and stroked over the surface of plates of neutral-red lactose agar.[2] The results were very uniform, as shown in table 1.

TABLE 1

	After 1 hour	After 2 hours	After 3 hours
Plates from surface of tubes containing 20 c.c. gelatin	Sterile	Growth	Growth
Plates from tubes containing 30 c.c. gelatin	do.	Sterile	do.

* The use of bile-salt, as a means of inhibiting the growth of non-intestinal organisms, has already become familiar, in MacConkey's[5] medium 'bile-salt agar.'

B. typhosus was the first to reach the surface in every case. On some of the plates a few colonies of *B. coli* were also present.

This seemed a promising line of investigation, and it was sought to still further simplify the procedure. One point in particular was encouraging; after keeping the tubes for some days there was no apparent diminution in the relative number of typhoid bacilli present in the fluid. This was contrary to what one had been led to believe previously, and was probably due to the fact that the medium was sugar-free, or practically so, making it impossible for *B. coli* to produce acid in it.

Experiment 2. 20 *per cent. Gelatin*

Artificial mixtures of typhoid and colon bacilli were prepared as before, and amounts varying from 0·1 c.c. to 1·0 c.c. were introduced into the bottom of each of twelve sterile tubes; 5 c.c. of 20 per cent. gelatin was then added to each tube and allowed to harden. Thus the capsules were done away with. After the hardening of the gelatin, 15 c.c. of bile-salt gelatin were introduced into six, and 25 c.c. into the other six, of the tubes, as before. Cultures were made from the surface at once, and after placing the tubes in the thermostat, at intervals of one hour, the results coincided almost exactly with those of Experiment 1, except that the simpler method, involving less jarring of the tubes, seemed to give the typhoid bacilli even a better chance of rising to the surface alone.

Experiment 3. *Isolation of B. typhosus from infected water*

As it was not possible to secure water which had been strongly suspected of contamination with typhoid faeces, 600 c.c. of tap-water were inoculated with two small loops of a young bouillon culture of *B. typhosus*, and small amounts of this infected water were introduced into the sterile tubes as in Experiment 2. Five c.c. of 20 per cent. gelatin were then introduced, mixed with the water, and finally 25 c.c. of 12 per cent. gelatin containing bile-salt were added as before. The results are shown in Table 2. The cultures were made as before, from the surface of the fluid in the tubes, after incubation.

TABLE 2

Showing the result of an experiment to detect, by the use of gelatin tubes, typhoid bacilli in infected water:—

Tube	Amount inoculated at bottom of tube	Plates made from surface of gelatin		
		At start	After 3 hours	After 22 hours
A	0·1 c.c.	Sterile	Sterile	Pure culture *B. typhosus*
B	0·2 c.c.	do.	do.	do.
C	0·5 c.c.	do.	Slight growth	do.
D	1·0 c.c.	do.	Sterile	do.
E	2·0 c.c.	do.	Slight growth	do.

Experiment 4. Isolation of B. typhosus from infected milk

A flask containing 200 c.c. of milk was inoculated with one loop of a twenty-eight hour bouillon culture of *B. typhosus*, and well shaken. Small quantities were introduced into tubes as in the case of the water; the tubes were filled with bile-salt gelatin and placed in the thermostat; cultures were made at intervals, with the results shown in Table 3.

TABLE 3

Showing the results of an experiment to detect, by the use of gelatin tubes, typhoid bacilli in infected milk:—

Tube	Amount inoculated at bottom of tube	Plates made from surface of gelatin		
		At start	After 2 hours	After 22 hours
N	0·05 c.c.	Sterile	Sterile	Pure culture *B. typhosus*
O	0·1 c.c.	do.	do.	do.
P	0·2 c.c.	do.	do.	do.
Q	0·5 c.c.	do.	do.	do.
R	1·0 c.c.	do.	do.	do.
S	2·0 c.c.	do.	Pure culture *B. typhosus*	do.

PRELIMINARY EXPERIMENT ON A METHOD FOR INCREASING THE NUMBER OF TYPHOID BACILLI IN A FLUID SUSPECTED OF CONTAMINATION

Four factors have been generally recognized as hindering the detection of *B. typhosus* in water, viz.:—(1) The comparatively small number present. (2) The lack of nutrition suitable to keep the organisms alive during the period of investigation. (3) The presence of *B. coli*. (4) The presence of other bacteria, chiefly saprophytes.

The previous experiment had led one to believe that under suitable conditions *B. typhosus* was not overgrown by *B. coli*. It was hoped, moreover, by the use of bile-salt and a high temperature to inhibit the growth of non-intestinal organisms in the water under examination. Two of the difficulties were thus met, theoretically at least. A suggestion recently made by Savage[9] led one to believe that the nutrition could be provided for; and finally, a knowledge of the method recommended by

Koch[7] for the easier isolation of cholera vibrios from stools, led to the following preliminary experiment* :—

Experiment 5. Three flasks A, B, and C were inoculated as follows :—

Flask A	Flask B	Flask C
Water, 2,000 c.c.	Water, 2,000 c.c.	Water, 2,000 c.c.
B. typhosus, $\frac{1}{1,000}$ loop.	*B. typhosus*, $\frac{1}{1,000}$ loop.	*B. typhosus*, $\frac{1}{1,000}$ loop.
	Peptone, 10 grammes.	Peptone, 10·0 grammes.
		Sodium taurocholate, 10·0 grammes

Remarks. Tap-water was used. One loop of a sixteen-hour bouillon culture of *B. typhosus* was inoculated into 100 c.c. of sterile water, and 0·1 c.c. of the mixture introduced into each flask. The peptone (Witte's) was introduced in concentrated solution and the same was done with the sodium, taurocholate.

The three flasks were placed together in the thermostat at 42° C. Two things were to be determined : (1) Whether the number of organisms was markedly greater in Flask B than in Flask A. (2) Whether the bile-salt prevented the growth of organisms other than in *B. typhosus* (Liverpool tap-water being daily shown to be free from *B. coli*) ; also, whether the introduction of the bile-salt apparently inhibited *B. typhosus*, so that there were less colonies of it on plates made from Flask C than on those made from Flask B.

After twenty-two hours' incubation, gelatin plates were made from each flask, 0·1 c.c. of the fluid being added to each plate. The writer was obliged to bring the experiments abruptly to a close before exhaustive determinations of the results could be made. After forty-eight hours' incubation, however, the plates showed the following results :—

Plates from Flask B showed a far greater number of colonies than those from Flask A.

Plates from Flask C showed less colonies than from Flask B. This was to be expected, because of the bile-salt in Flask C. Time did not suffice to determine whether the number of colonies of *B. typhosus* on plates from Flask C was less than that on plates from Flask B. Sub-cultures made from the colonies of the former proved them to be without exception, however, those of *B. typhosus*.

In conclusion. The experiments here reported are neither numerous enough

* Experiments conducted by Vaughan[11] showed that, by incubation at high temperatures, water organisms could be excluded.

nor extensive enough to warrant any large general deductions. The results were uniform enough, however, to be summarized as follows :—

1. The motility of *B. typhosus* is enough greater than that of *B. coli*, so that if infected material be introduced into the bottom of sterile tubes, temporarily confined there, and then liberated into a long column of fluid, preferably a viscid one, *B. typhosus* will regularly rise to the surface first, and can there be readily isolated, often in pure culture.

2. The presence of sodium taurocholate in the fluid used arrests the activity and development of organisms other than intestinal, especially if incubation be at 42° C.

3. If non-intestinal organisms are thus suppressed, and the medium be free from sugar, the presence of *B. coli* will, probably, not interfere with the development and detection of *B. typhosus*, in case the latter be present.

4. It seems likely that if to fairly large quantities of suspected water there be added a small percentage of peptone, or other suitable sugar-free nutrient medium, as well as a concentrated solution of bile-salt, and the mixture be incubated for twenty-four hours at 42° C., the chances of isolating *B. typhosus* will be greatly increased.

September, 1902

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THE THICK-FILM PROCESS FOR THE DETECTION OF ORGANISMS IN THE BLOOD

THE THICK-FILM PROCESS FOR THE DETECTION OF ORGANISMS IN THE BLOOD

By RONALD ROSS

IN the *Journal of State Medicine*, December, 1902, and the *Lancet*, January 10, 1903, I described a method which facilitates the detection of parasites of the blood by enabling us to examine thick films of dried blood in place of the thin films now generally used for the purpose. Instead of spreading a small quantity of blood over a large area and then fixing the haemoglobin and staining, the proposed method consists in taking a comparatively large quantity of blood, scarcely spread out at all over the slide, and stained without *fixing the haemoglobin*. In the first method one is often compelled to search many fields before finding a parasite: in the second method the parasites are much more crowded together owing to the thickness of the film of dried blood employed; and are just as visible as in the thin film owing to the fact that the haemoglobin, the opaque element of the blood, not being fixed, has been washed out during the process of staining with aqueous stains.

Photographs of a preparation of malarial blood made by this method were published in the *Journal of Tropical Medicine*, February 2, 1903; and Ruge has verified and amplified the process in the *Deutsche Med. Woch*, March 19, 1903, S. 205. Recently Bell and Laing advocate it for the detection of plague bacilli in blood (*Lancet*, June 20, 1903); and I am confident that it will be found useful for diagnosis in many affections besides malaria.

I now give a water-colour drawing of a field of a thick-film preparation of blood taken from an ordinary case of aestivo-autumnal infection. In a thick-film preparation of the same blood, less than one parasite on the average could be found in each field; whereas it will be seen that about eighty parasites can be counted in the field given in the plate. The photograph shows a field of a thick-film preparation of rat's blood containing *Trypanosoma brucei*. In a thin film of the same blood only one or two parasites could be found per field.

I am now using the following stock formulae for staining :—

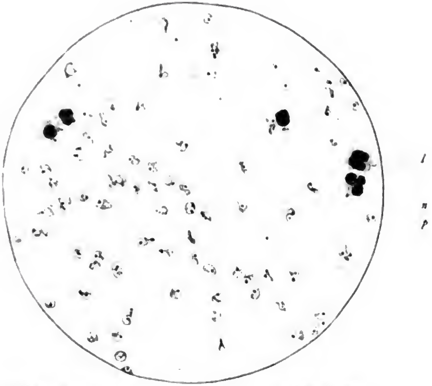
Eosine, 1 gramme	Medicinal Methylene Blue, 10 grammes
Water, 1,000 c.c.	Sodium Carbonate, 5 grammes
	Water, 1,000 c.c.

It is necessary to keep the blue solution for some days in a warm place until it has acquired the well-known purple tint. The thick film of blood on the slide is dried, and then very gently washed until all the colour has disappeared from it. A drop or two of the eosine solution is now placed upon it (without previous drying), and allowed to remain for a minute. Next, a drop or two of the blue solution is run in, and allowed to remain from fifteen to thirty seconds. Lastly, wash with a very gentle stream of water for a minute or so; dry, and mount or examine.

The student must practice the method in order to obtain perfect results. He should aim at staining the parasites in a typical manner without staining the stromata of the red corpuscles. If the preparation is so much overstained that the stromata are deeply coloured, the preparation is not so elegant, though it may still be useful.

For crescents and other large pigmented malarial parasites, I use the weakest possible staining, sufficing just to give a faint tint to the organisms without obscuring their melanin. With trypanosomes the blue must be applied somewhat longer than for malaria parasites. It should be noted that for *Haemamoeba vivax* the method has not proved very satisfactory owing to the indecisive staining of the chromatin.

It is advisable to use a high ocular, say Nos. 4, 8, for finding the smallest organisms.



G. S. Boston, del. 1903 *Mag.* 750 *diam.*

Young Amoebulae of *H. praecox* in Thick Film
l, leucocytes. *p*, parasites. *n*, nucleus of parasites

Mag. 250 *diam.*
Trypanosoma brucei of rat in Thick Film

NOTE ON THE STAINING OF BACTERIAL FLAGELLA WITH SILVER

NOTE ON THE STAINING OF BACTERIAL FLAGELLA WITH SILVER

By J. W. W. STEPHENS, M.D., Cantab
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IN a short communication to the *Lancet*, 1898, I stated that by using largin, an albuminate of silver, I had easily been able to obtain well-stained and clear specimens of flagella. I observed at the time that this body, largin, smelled distinctly of ammonia, and I suggested that my success in using this modification of Van Ermengem's well-known method was due to this cause. I was unable at the time to pursue the matter further, and left it to any other person interested in the subject to carry out. So far as I know no one has pursued the subject in this direction, and it is only quite recently that I have again taken the matter up.

I will not here give all the many experiments I made, suffice it to say that it soon became clear that the advantage in using largin depended not upon its albuminate but upon its ammonia content.

So that I next experimented with ammoniacal silver solutions, but found that it was more convenient to add the ammonia to the tannic and gallic acid solution of Van Ermengem, because ammonia precipitates this acid solution, and by dissolving the precipitate in excess of ammonia a clear solution is got. With this ammoniacal solution I was able to get beautifully stained specimens.

I next proceeded to find out which constituent of Van Ermengem's somewhat complex mixture was the essential one, and I found that an ammoniacal solution of tannic acid alone gave as good results as Van Ermengem's mixture. (So far as I have experimented, gallic acid alone has not given me positive results). It was next a question of determining the best proportions of tannic acid and ammonia respectively, and after numerous trials I find that good results can be constantly got in the following way, though I do not assert that I have yet found the best proportions :—

I take no extraordinary precaution in cleaning glass sides—for the whole of the staining is most readily done on slides—except that after cleaning with a pocket-handerchief I heat the slides thoroughly on a clean piece of wire gauze over the bunsen.

The emulsion is made in the ordinary way, or for simply determining whether or no a bacterium has flagella I smear a mass of culture, moistened with water, over the slide in the same way as I would make a blood smear with a needle. So far I have used VAN ERMENGEM's mordant osmic acid and tannin; I have, however, got positive results by using tannin alone after mordanting for some days, but have not made many experiments in this direction. I use then the following solutions:—

1. VAN ERMENGEM's mordant of osmic acid and tannin, one-half to one hour
2. A 0·1 per cent. solution of silver nitrate
3. 5 per cent. tannic acid + 5 per cent. NH_3 solution *(partes aequales)*

Wash off the mordant thoroughly with tap-water.

Pour some of the silver solution with a pipette over the slide, then add a few drops of the ammonium tannate solution till a deep reddish-brown colour is produced, and allow to stain for a minute or so, or as long as no black precipitate is formed.

Wash off in ordinary tap-water.

Repeat this procedure two or three times until the film has a deep brown or somewhat black colour.

It is usual in describing a new method for staining flagella to claim that the preparations are most beautiful and quite free from precipitate, without asserting this, I will simply say, that in my hands this method has given, with great ease, good preparations.

A PRELIMINARY NOTE ON THE SUPPOSED BACTERICIDAL INFLUENCE OF FLOUR AND ALLIED SUBSTANCES ON BACILLUS TYPHOSUS

A PRELIMINARY NOTE ON THE SUPPOSED BACTERICIDAL INFLUENCE OF FLOUR AND ALLIED SUBSTANCES ON BACILLUS TYPHOSUS

By HERBERT E. ROAF, M.B. (Tor.)
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THE following experiments were performed to follow out some results obtained by Klein and Houston[1] :—Both these observers used media composed of flour, oatmeal, and ground rice in a ten per cent. emulsion. The media were inoculated with various bacteria, and inoculations were made from them at definite intervals. According to the length of time that the bacteria could be demonstrated it was judged if there was any bactericidal effect. No mention was made, however, of control experiments with sterilized water. They found that in a wheat-flour medium, *B. typhosus* was absent after three to five days ; *B. diphtheriae* after one day ; *S. pyogenes aureus* after thirteen to twenty-four days ; *V. cholerae* after three to six days, and *B. pyocyaneus* after fourteen days. That in oatmeal, *B. typhosus* was absent after six days ; *B. diphtheriae* after one day ; *V. cholerae* after two days ; and *B. pyocyaneus* after four days. In ground rice, *B. typhosus* was absent after twenty-five days ; *B. diphtheriae* after three days ; *V. cholerae* after two days, and *B. pyocyaneus* after twenty-nine days.

In the following series of experiments I employed the *B. typhosus*. The media consisted in most cases of a ten per cent. emulsion of wheat flour. In some of the experiments the material was filtered through a sterilized Pasteur-Chamberland filter in order to free it from contaminating organisms.

In two experiments the filtered juice obtained from potatoes and apples was used, and lastly, sterilized water was employed as a control. The medium was in each case inoculated with a pure culture of *B. typhosus*, and a culture was immediately made using one c.c. of the mixture. This was either plated immediately or diluted to some sub-multiple of ten and one c.c. of the dilution used. The material used for plating was neutral red taurocholate lactose agar[2]. The plates were incubated, and after twenty-four hours the white colonies were counted. If the colonies were too numerous to be counted they were marked thus ∞.

1. *Report on the Behaviour of Specific Microbes in Relation to Cereal Products. Local Government Reports*, 30th Annual Report.
2. Grünbaum and Hume, *British Medical Journal*, 1902, I, p. 1473.

The various inoculated media were kept in a dark place at room temperature, and from day to day one c.c. undiluted, or diluted if necessary, was plated. After the colonies were counted, subcultures were made in DURHAM's fermentation tubes containing glucose broth. The following experiments are selected from a large number which gave similar results.

The growth of the *B. typhosus* in ordinary flour was hard to estimate, owing to the overgrowth of contaminating organisms, but in one or two cases, where the contamination was of slight degree, results similar to those of KLEIN and HOUSTON were obtained.

Experiment 1. Ten grammes of flour were weighed out, and 100 c.c. of sterilized water added. This was kept in a sterilized flask and inoculated with *B. typhosus* :—

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 100,000	132	13,200,000
1 day	1 : 1,000,000	2,226	2,226,000,000
4 days	1 : 1,000,000	1	1,000,000
5 days	1 : 10,000	0	Less than 10,000
7 days	1 : 1	0	0
8 days	1 : 1	0	0

To avoid the difficulties caused by contamination, another series of experiments were undertaken to see if filtered solutions would give the same result.

Experiment 2. A ten per cent. mixture of flour in sterilized water was made. This was filtered through a sterilized PASTEUR-CHAMBERLAND filter and inoculated with *B. typhosus* :—

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 10,000	1,080	10,800,000
1 day	1 : 100	1	100
3 days	1 : 1	0	0
5 days	1 : 1	0	0

Experiment 3. Conditions exactly the same as in experiment 2.

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 10,000	282	2,820,000
1 day	1 : 100	0	Less than 100
3 days	1 : 1	2	2
5 days	1 : 1	0	0
6 days	1 : 1	0	0

In these experiments the *B. typhosus* disappears more rapidly than in the unfiltered emulsions. The reason may be that the filtering removes some of the nutriment contained in the mixture of flour and water.

An attempt was next made to see if flour could be sterilized and yet possess any bactericidal effect. Ten grammes of flour were sterilized in flowing steam for twenty minutes on three successive days. One hundred cubic centimetres of sterilized water were added to the ten grammes of sterilized flour, and the mixture was inoculated with *B. typhosus*. The experiment is too long to give in tabular form, because there was no decrease in the number of organisms for thirty-three days, and it was recovered as late as the 127th day after inoculation.

Two explanations might be given of this result :—

1. The heating may have destroyed the bactericidal substance.
2. The heating may have changed the flour so that it formed better food for the bacteria.

To throw some light on this problem, a series of experiments were made by filtering a ten per cent. emulsion of flour in sterilized water through a sterilized Pasteur-Chamberland filter. The filtrate was heated to boiling, and inoculated with *B. typhosus* (see experiments 4 and 5).

Experiment 4

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 1	∞	∞
2 days	1 : 1	20	20
4 days	1 : 1	0	0
5 days	1 : 1	0	0

Experiment 5

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 1	∞	...
2 days	1 : 1	0	...
4 days	1 : 1	0	...
5 days	1 : 1	0	...

These results appear to disprove that the bactericidal power, if it exists, is destroyed by heating.

A preliminary analysis of these media gave the following reactions :—

On heating, a light precipitate was thrown down.

Acidity 10 c.c. = 0·15 c.c. $\frac{N}{10}$ NaOH.

Fehling's test showed a trace of a reducing substance present.

Biuret reaction showed the presence of proteid.

A precipitate was obtained by saturating some of the solution with ammonium sulphate. This precipitate was washed and dissolved in sterilized water.

The solution so obtained was inoculated with *B. typhosus.* The bacillus disappeared in from eight to nine days, thus showing no special bactericidal effect. Two experiments were made with potato juice filtered through a sterilized filter and inoculated with *B. typhosus.* One was heated to boiling before inoculation. In both these the growth was very luxuriant after eighteen days, when the material was exhausted. Similarly, apple juice had no bactericidal effect.

As a control, a series of experiments were made by inoculating *B. typhosus* into sterilized tap water.

Experiment 6. Sterilized water inoculated with *B. typhsous.*

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 100	∞	∞
1 day	1 : 100	17	1,700
3 days	1 : 1	∞	∞
5 days	1 : 1	12	12
6 days	1 : 1	10	10
7 days	1 : 1	5	5
8 days	1 : 1	0	0
10 days	1 : 1	0	0

Experiment 7. Sterilized water inoculated with *B. typhosus*.

When cultures were made	Dilution	No. of colonies on plate	No. of bacteria per c.c.
Immediately	1 : 1	∞	∞
2 days	1 : 1	0	0
3 days	1 : 1	0	0

The work has had to be interrupted. It remains for further investigation to show precisely to what extent a constituent of flour is responsible for the inhibitory action, or to what extent the decrease in nutriment determines the diminution in the number of bacilli.

In closing, I wish to thank Dr. GRÜNBAUM, who suggested this line of research.

THE RELATION OF VESICULAR MOLE TO
CHORION CARCINOMA

THE RELATION OF VESICULAR MOLE TO CHORION CARCINOMA

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BEFORE entering upon a discussion of the pathology of vesicular mole and its frequent sequaela, chorion carcinoma, it is necessary to review, somewhat in detail, the opinions at present held concerning the development and structure of the chorionic vesicle.

Much light has of recent years been thrown on this difficult subject by the discovery of several observers of certain very early ova.

Foremost among these is Peters, of Vienna, who, in 1899, secured a human ovum of only two or three days' growth, measuring 1·6 by ·8 and ·9 millimetres in diameter; so far as is known the youngest ovum on record. The description of this and of the way in which it was embedded in utero has, to a great extent, revolutionized the hitherto widely accepted views of human placentation.

Of the many theories held concerning the mode of attachment of the fertilized ovum to the uterus, the following may be mentioned:—

1. That the ovum, having passed through the first stages of segmentation immediately following fertilization, becomes speedily embedded in the soft and thickened mucous membrane, which is soon reflected completely over it, the ovum coming to lie in a cavity shut off from the general cavity of the uterus.

2. That some abrasion of the uterine mucosa was essential to the formation of a suitable resting-place for the ovum.

3. The opinion expressed by Berry Hart that possibly the ovum grafted itself on the connective tissue of the uterus which had been exposed by the removal of the mucous membrane during menstruation. 'Pfluger first suggested that the human ovum can only be implanted on connective tissue, and thus cannot develop in the healthy tube or cervix, and the view has been strongly upheld by Lawson Tait. This theory accounts for the frequency of conception just after menstruation, and for the fact that tubal pregnancy is usually preceded by disease destructive of the tubal epithelium, but it does not account for those cases of tubal gestation with no previous tubal disease, nor for the occurrence of uterine gestation remote from menstruation, as during lactation and pathological amenorrhoea.' (Fothergill).

As an alternative hypothesis, it was suggested by HART that the fertilized ovum had the power of making its way through the uterine epithelium, as has been observed in the development of the guinea-pig. (HUBRECHT).

This latter view is now thought to be the true one, especially since it has been confirmed by the observations of PETERS, who maintains that the epiblast of a fertilized ovum has an inherent phagocytic action, and by its means is able to attach itself to the maternal wall by burrowing downwards and laterally into the mucous membrane, and so coming to lie embedded in a cavity of its own formation.

The following is a brief summary of the description of the early ovum from which the above theory was deduced :—

PETERS found the ovum on the second or third day, lying in a deep cavernous space with overhanging walls, in which no maternal epithelium was seen. Except at the point of entrance to the cavity where a plug of fibrin occupied the opening, the epithelium on the surface of the mucous membrane was completely intact. The rest of the mucosa was found to be congested and oedematous, with some degeneration of the epithelial cells in the neighbourhood of the ovum, while in the deeper layers there was slight hypertrophy of the glandular elements. Decidual cells were first noted at some little distance from the ovum, being formed by the enlargement and rapid proliferation of the connective tissue cells of the stroma.

The ovum is described as containing an embryonic area composed of a few cylindrical cells, an amniotic cavity completely closed, lined in the outer portion by flat cells, and surrounding all the chorionic vesicle. Between the amnion and the chorion there were several layers of mesoblast, the amniotic cavity being, in fact, embedded in mesoblast. The chorionic vesicle was lined by a thin layer of mesoblast, a slight space, however, separated it from the outer layer, which consisted of an irregular meshwork of epiblastic cells, forming a delicate reticulum between the ovum and the tissue in which it is embedded. The spaces in this network were filled by maternal blood.

It is important to emphasize the fact that the earliest chorionic epithelium is cellular and not plasmodial in nature, as usually described. For it the name 'trophoblast' has been suggested by HUBRECHT, who speaks of it thus :—

'The first new name of which I want definitely to establish the significance is the name trophoblast. I propose to confer this name on the epiblast of the blastocyst, as far as it has a direct nutritive significance, as indicated by proliferating processes, by immediate contact with maternal tissue, maternal blood, or secreted material. The epiblast of the germinal area, the formative epiblast, and that which will take part in the formation of the inner lining of the amnion cavity is, *ipso facto*, excluded from the definition.'

As indicated in the above definition, these cells take no part in the formation of the embryo, but they serve to anchor the ovum to the maternal tissues, and act as

a means of nourishment by the absorption by osmosis of certain elements from the maternal blood in which it is bathed. This cellular condition of the trophoblast is only seen in the very earliest stages, and, as shown in the above specimen, is soon replaced by the plasmodial form, especially in the outer layers of the reticulum where the process first begins. In these places the cells were seen to have lost their definite outline.

It has been suggested that this is due to the pressure of the blood, and to some chemical action of its plasma, and it is not difficult to recognize in these cellular masses the syncytium of the fully formed villus.

Peters also noticed that the trophoblast was somewhat more abundant on the side facing the decidua basalis than on the outer side. Even at this very early stage the differentiation of the outer layer of the chorionic vesicle into villi had begun, and this precocity of development of the human chorion has been one of the greatest factors in misleading embryologists, who have based their observations on the conditions found in the chick.

Coste, in 1847, was the first to observe the rôle of the epithelium of the chorionic vesicle. In his description of an ovum, fifteen to eighteen days old, he shews the chorion to consist of two layers: an inner membrane passing continuously over the inner surface of the chorion, and an outer, which alone formed the hollow villi, so that in looking on the inner surface of this layer, numerous small openings were seen which did not penetrate the inner membrane. Coste believed these villi to be the pathfinders for the blood vessels of the fully formed villi serving temporarily to anchor the ovum to the uterus, but he did not describe the histological appearance of the layers. Kolliker, in 1861, had an opportunity to examine the chorion, and he found that the outer membrane was epithelial, with cells of the same character as in the epithelium of older vascularized villi, and that the inner layer consisted of developing connective tissue and carried fine blood vessels. (Minot).

In Schawbe's embryo, thirteen to fifteen days old, the chorionic villi were considerably branched and entirely filled with mesoderm. Their tips had little thickenings of epithelium by which they were attached to the decidua, and this was the only connexion between the foetal and maternal tissues.

According to Minot, the reason for this is that the uterine mucosa does not grow in between the villi, nor do the villi penetrate the cavities of the uterine glands, 'only the tips of the villi touch the surface of the decidua either at first or subsequently, except, of course, over the chorion laeve during the abortion of the villi. The tips of the villi are attached to the uterine surface, they penetrate the decidua for a short distance, but even in the placental area at the close of gestation the penetration is slight and the villi make their way only into the surface stratum of the decidua serotina. There is no evidence of any sort that the villi penetrate the glands at any period.

The very slight attachment of the ovum to the decidua thus seen has also been demonstrated by FOTHERGILL, who described a very early ovum which was expelled with a decidual cast.

The ovum, which was a small yellow body, about the size of a 'split pea,' was found on a plug which had been used to pack the vagina, but no trace of its resting-place was to be seen in the cast.

Sections of the decidua showed the usual typical structure. The uterine epithelium remained unaltered in the deep layer of the decidua, but the glands were dilated and had lost their epithelium in the middle and superficial layers. There were large decidual cells and numerous spaces filled with fresh blood. No chorionic villi or portions of chorionic epithelium were visible.

Examination of the small yellow body showed a complete ovum about a half centimetre in diameter, consisting of a sac of chorion only, completely covered with chorionic villi. No traces of an amnion or germinal area were found. FOTHERGILL believes that this specimen had not grown for more than five or six days.

In October, 1901, a similar cast was handed over to me for investigation, which proved of great interest, in that it revealed an ovum, corresponding in many respects to that of PETERS. The specimen (see Fig. 1, Plate VIII) shows a very perfect decidual cast. It has three openings, one corresponding to the os, the remaining two to the openings of the Fallopian tubes. The anterior surfaces had a flattened appearance, while the posterior was rounded in contour, the whole being shaggy and slightly nodular. It had been spontaneously expelled from a patient who had missed one period and who was suffering from retroflexion. On examining the external appearances of the specimen, attention was first called to the anterior and upper surface by a slightly pale area. This was carefully incised and found to be the site of a complete ovum, embedded in the decidual tissue. It measured 5 mm. in its longest diameter and 4 mm. in its shortest.

On section, the decidua appeared very much the same as that described in FOTHERGILL's case, and enclosed the ovum completely, except at one point only, demonstrable under the microscope, where the decidua capsularis was wanting. (It is believed that, at this point, the fertilized ovum burrowed its way into the maternal tissue). Well-formed villi are seen surrounding the chorionic vesicle (see Figs. 2 and 3, Plate VIII), but even at this early stage they are more numerous at the point opposite the decidua basalis. The epithelium covering the villi has, in many places, retained its primary trophoblastic form, in this respect, closely resembling the cells found in PETERS' ovum, and is seen forming a distinct connexion between the ovum and the maternal wall. In most of the villi, the trophoblast forms a single investing covering, which shows the syncytium with numerous buds in the outer layer, and a more definite inner row of cubical cells, the 'Zellschicht' of LANGHANS. In several of the sections a distinct embryonic area is seen, composed of an irregular mass of

Fig. 1
Decidual Cast with Ovum

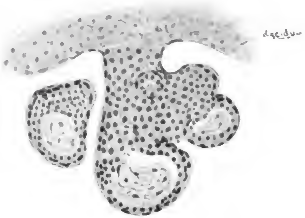

Fig. 2
Trophoblast Cells and early Villi

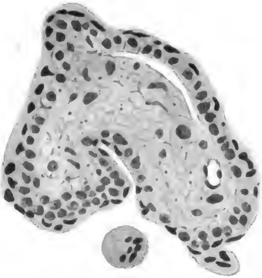

Fig. 3
Fully formed Villus with Syncytial Buds

Fig. 4
Decidual Cells

epithelioid cells, having a very slight attachment at one point only to the chorionic vesicle. Close to this area, and apparently in contact with it, is a small cavity, believed to be the amnion.

(It may be noted, in passing, that from the appearances found in the above specimen, the statement made by PETERS, that the amnion does not form by free folds as has hitherto been taught, but by an inclusion of epiblastic cells in the form of a plug, by the breaking down of which the embryo, body cavity and amnion are formed, receives confirmation).

It is a significant fact that at no point in the decidua which the chorionic villi had not touched was there any syncytial tissue, a point which has been much emphasized by MINOT in discussing the foetal origin of the syncytium.

That the early ovum is thus only anchored and not adherent to the maternal tissues was also demonstrated in another specimen which came under observation.

A small ovum, measuring about 4 c.m. in diameter, almost completely surrounded by decidua, was expelled by a patient who had missed one menstrual period.

This was floated out in water, and with great care the decidual tissue was gently stripped away. No force was used or required, the villi detaching themselves without any apparent damage, the ovum then presenting the appearance of a shaggy spheroid. The amnion and chorion were separated and pieces were stained and mounted on the flat. The amnion consisted of two distinct layers, the inner being formed of flat epithelial cells with oval nuclei, many in a state of active division (epiblast); the outer of young connective tissue, long spindle and branching cells varying greatly in size and shape (mesoblast). The chorion, with which we are chiefly concerned, also consisted of two layers, the inner mesoblastic layer corresponding with that seen in the amnion. The outer layer was also formed of flat epithelial cells, but here the cells were of more than one layer, two were seen in some places, but the arrangement was not uniform. Here and there over the membrane buds of the outer layer were seen, some branched giving off smaller buds and having a mesoblastic core, others apparently hollow buds of epithelium. The tips of the villi were found to be quite complete, ending in each case by a solid mass of cells, giving to the villus a clubbed appearance.

Sections were then cut of the decidua (see Fig. 4, Plate VIII). These shewed typical decidual tissue, flat, polygonal spindle and round cells with large round nuclei. The free surface to which the ovum had been attached was reticulated, deep recesses and spaces running up into the compact layer, but the importance which is attached to the specimen consists in the fact that no trace of foetal structure, such as syncytial cells or other parts of villi were seen in any of these spaces, some of which were filled with blood, or in any other part of the section, and it is not unreasonable to conclude that in these spaces, bathed in maternal blood, the clubbed ends of the villi were previously situated.

Having thus briefly described the appearance of the chorionic vesicle in the early ovum, the theories concerning the origin of the different layers must be considered. This is of particular importance in view of what follows in the discussion of chorion carcinoma or deciduoma malignum so called.

For many years a controversy has been waged by pathologists, Continental, American, and British, on this subject, without any consensus of opinion having been arrived at.

On one point, however, all observers are agreed, namely, on a mesoblastic origin for the central core of the villus, but it is the epithelial or external covering to this core which has given rise to so much discussion.

In a fully-formed villus of the second or third week we find an outer plasmodial layer, composed of highly refracting granular protoplasm, taking on a deep rich stain with eosin, having no cell walls and well supplied with vacuoles. In it are seen numerous darkly-stained nuclei, of various sizes and shape; some are elongated and flattened as though subjected to pressure from without, and lie scattered throughout the protoplasm, others are crescentic or triangular, while others again are oval or round. These may lie massed together in great profusion, or they may, in places, be very sparsely distributed; this layer is the syncytium. In direct contact with the foregoing is another layer, composed of more transparent cubical cells, having a definite cell outline, containing a single nucleus and separated by a slight interval from the mesoblast forming the central core. To this layer little attention was paid by early observers, and it was not until 1882 that LANGHANS published his classic description of the *Zellschicht.*

Speaking of the distinctive points between this layer and what he called the epithelial (syncytial) layer, he says: 'The "zellschicht" consists for the most part of polyhedral sharply defined cells, whose lines of division can in most places be distinguished, except where the layer is thin, containing clear, almost transparent protoplasm, poor in granules, with large, spherical, seldom flattened nuclei and usually a nucleolus.' LANGHANS further emphasizes the variety of forms these cells may assume, from tall cylindrical rods to small spherical or flattened cells, according to the thickness of the layer and the pressure to which it is subjected. Of the syncytium he writes, that it lies closely over the 'zellschicht,' filling up all depressions in its upper surface, and sending down processes in between the cells, giving one the impression that there is here a double layer of epithelium rather than two layers of really different tissues.

LANGHANS thus believes that the epithelial layer and the 'zellschicht' are derived from two distinct sources, since he plainly states that the 'epithelial' layer is derived from the foetal ectoderm, while the 'zellschicht' has its origin in the connective tissue cells of the stroma of the villus, and is, therefore, of mesoblastic origin.

This represents the view of most English and Continental observers.

CULLEN refers to it as follows: 'It is doubtful whether LANGHANS' layer in reality consists of epithelial cells, some believing that it probably represents nothing more than the outlying stroma cells, which have of necessity assumed a marginal arrangement.'

ROBERTS follows EDEN in saying that LANGHANS' layer is a 'specialized stratum of the connective tissue corpuscles of the stroma.'

There are, however, several observers whose opinion must carry great weight who differ very widely from the foregoing. Among these are MINOT, KATSCHENKO, KUPPFER, SPEE, WEBSTER, and PETERS, who all believe not only in a common foetal origin for both syncytium and 'zellschicht', but also that both layers are derived from the foetal ectoderm. It is of interest that, after careful microscopic study of the young ovum previously referred to, I had independently arrived at the same conclusion.

Turning now, for a moment, to the consideration of the origin of the syncytium, we find that a still greater diversity of opinion exists.

Many believe it to be an entirely maternal product.

TURNER believes the whole covering of the villi to be derived from the uterine epithelium.

ERCOLANI derives it from the connective tissue of the uterus, *i.e.*, from the decidual cells.

WINKLER agrees that the covering has two layers, but derives the inner from the foetal epithelium, and the outer from the endothelium of maternal blood sinuses.

MINOT, KUPPFER, SPEE, HART, GULLAND, MERRTENS, and FOTHERGILL, all believe that the syncytium is derived from the foetal ectoderm.

WEBSTER says, 'the foetal origin of the syncytium cannot now be denied. It has been clearly pointed out in different manuals by VAN BENEDEN, HUBRECHT, and DEWAL. The importance of the labour of these authorities in the investigation of this matter cannot be too highly emphasized. Among recent German workers, their work has received scant attention. The maternal origin of the syncytium was advocated in the days when there was no such careful technique in microtomy as is found now-a-days, and it is hard to break away from traditional views.'

KATSCHENKO believes in a common ectodermal origin for the zellschicht and syncytium, and ULESCO-STROGANOWA says, 'One can undoubtedly see an intimate connexion between the cellular elements and the syncytium,' and firmly believes in the same 'genetic identity' for both layers.

MINOT has shown very clearly, in his elaborate description of the human chorion, that both epithelial layers of the villus are already formed before the connective tissue core has penetrated to its tip. 'They are at first clumsy cylinders, which may grow to a millimetre in length before they begin branching. They arise,

as shown long ago by Coste, as outgrowths of the ectoderm only,' and concludes by saying, 'there is, in my judgment, no reason left for differing from the conclusion that both layers are parts of the foetal ectoderm.'

As most of the foregoing opinions were based on observations made from ova of the second or third week, when the villi are fully formed, it is not surprising that so much diversity of opinion existed between different observers, and one is convinced that it is only by a careful and minute investigation of the earliest possible ova, that a true idea can be formed of the development of these layers.

Peters, in his monograph, *Ueber die Einbettung des menschlichen Eies*, previously referred to, points out that in the earliest stages the cavity of the ovum is surrounded by a thick cellular layer, the trophoblast. This is made up of one complete mass of cells, broken up here and there by more or less large blood spaces.

Peters shews that these spaces communicate with each other by small connecting canals, which he has been able to trace in a number of serial sections. These spaces are filled with maternal blood, and they may again be seen to communicate with the maternal sinuses in the decidua. The innermost cells of the trophoblast are cubical in shape, and possess oval or round nuclei. They form a continuous layer of cells next the mesoblast, but at this early stage are separated from the connective tissue in most places, by a narrow space, giving the impression that the mesoblast has not yet followed the epithelial mantle in all its outgrowths. Between this layer and the uterine mucosa, the cells are arranged in groups and strands, and correspond in every respect to the cells forming the inner layer, except in those places where they are in contact with the maternal blood.

Here they are seen assuming a flattened shape, the nuclei tend to become spindle or rod shaped, in some places crescents are seen, and here and there a dense mass of nuclei, stained very deeply, giving the appearance under the low power of one gigantic nucleus.

In my own specimen, which has been already described, the trophoblast can still be recognized as forming a connecting link between the ovum and the maternal tissues. The cells are more polygonal than those described by Peters, but they correspond entirely with the inner layer of epithelium of the villi, the 'zellschicht' of Langhans, and are seen to be a direct continuation of the cells forming that layer.

In some of the villi only a single cubical row of cells is seen below the flattened outer layer, but in quite a number, multiple row of cubical cells are present.

It is specially noticeable in this specimen, as in Peters, that the mesoblastic core is not yet in direct contact with the epithelium, but is separated from it here and there by slight spaces.

From the consideration of these points we can easily recognize in the outer flattened layer of the trophoblast, the syncytium, and in the inner cubical layer, the

'zellschicht' of LANGHANS. The trophoblast in its primitive form can only be seen in the very early ova of the first weeks of pregnancy. I have never been able to find any in ova of more than a month old. In one specimen I have examined, of about three weeks old, the transition from trophoblast cells to syncytium is well seen. Three layers of cells cover in the central core of the villus, the outer layer is completely flattened, the nuclei spindle shaped and crescentic. The protoplasm, in which these nuclei lie, stains well with eosin and forms a continuous ground work for the second layer of nuclei, which are larger and more oval in shape, possessing numerous granules, but staining less deeply than the outer layer. The third or innermost layer has the same continuous protoplasmic basis, and, lying in it, are large round nuclei, having a somewhat transparent appearance, possessing fewer granules and arranged in a regular manner round the stroma of the villus.

Writing on this subject, PETERS says: 'I have always found, in all my preparations, an intimate and genetic connexion between the 'zellschicht' and syncytium, and I was never able to demonstrate a limiting membrane existing between the two layers.'

I, therefore, regard the whole epithelial covering of the ovum, including that of the villi, as foetal and ectodermal, and consider those theories which infer a maternal origin for the syncytium and a mesodermal origin for the 'zellschicht' as fallacious, and for the following reasons:—

1. The syncytium cannot be formed from the epithelium lining the glands of the uterine mucosa, since these have been seen to possess no connexion with the ovum in the earliest stages, their epithelium being quite intact, and at no point invading or being invaded by the foetal trophoblast.

2. The syncytium cannot be derived from the endothelium of the maternal blood vessels, as it has been proved by many observers that this shows no sign of activity during the early weeks of pregnancy.

3. The syncytium is not derived from decidual cells, as in PETERS' ovum the connective tissue of the stroma were only beginning to take on a distinct change at some little distance from the ovum.

4. The 'zellschicht' of LANGHANS is not a specialized stratum of the connective tissue cells of the stroma, since it has been proved by MINOT, PETERS, and others that this layer is already well seen before the mesoblastic core of the villus has penetrated completely into it.

As will be seen later, these layers of chorionic epithelium take an active part in the formation of vesicular mole, and a still more active and important part in the production of chorion carcinoma, so that in discussing the pathology of this latter condition, it is of the utmost importance that the foetal and ectodermal origin of these cells be kept in mind.

PART II

The chorionic vesicle is, in all stages of its existence, liable to undergo hydatidiform degeneration (Fig. 5, Plate IX). This condition, about which so little is known, is of great interest, owing to the striking connexion it has been seen to possess with chorion carcinoma, and as an understanding of the clinical features is essential to the true interpretation of the pathological appearances, a few cases, which, through the kindness of Professor Briggs, of the University of Liverpool, have come under my observation, will be discussed.

According to the date of pregnancy at which the disease appears, the chorion may be affected.

1. In its whole extent, in which case the mole presents an ovoid shape, corresponding closely with the interior of the uterine cavity, composed of vesicles varying greatly in size, having no trace of a foetus or amniotic cavity.

It may or may not have an enveloping membrane of decidua. These cases originate in the early weeks of pregnancy, and form the commonest variety of the disease.

2. In an area corresponding to what should have been the placenta. In these cases the mole is more compact ; there may be the remains of a foetus inside a well-defined amniotic cavity. This class arises during the first and second months of pregnancy.

3. In a limited area of what appears otherwise as a normal placenta. Here the foetus may be quite healthy and well developed, the pregnancy having gone on to full term without an untoward symptom occurring. This class of cases is extremely rare, as are also those cases in which a twin pregnancy exists, one ovum being a living child and the other a vesicular mole. The literature bearing on these cases is very scanty, and, beyond the fact of their occurrence being recorded, little more is known of their pathology.

The etiology of this condition is obscure, and many factors must be taken into consideration in trying to arrive at the true cause of the disease.

The age of the patient is apparently of little importance, as the disease may occur at any time during the child-bearing period, cases having been recorded as early as seventeen and as late as fifty-three. It has, however, been noted that the condition is more frequent in multiparae, especially in those who have borne few children, and in those cases where the pregnancies have not followed each other with great rapidity.

Although most writers agree in assigning as a predisposing cause, some pathological state of the mother, some have suggested syphilis in the father as a possible cause.

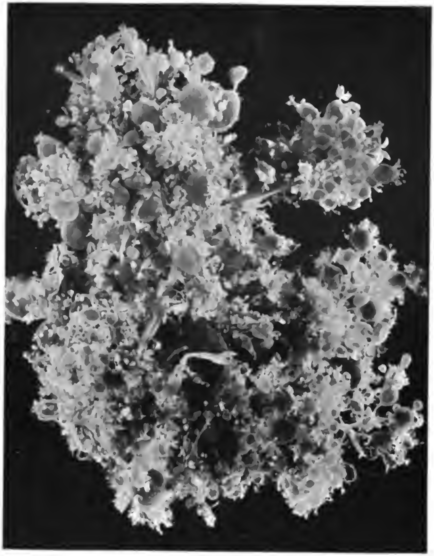

Fig. 5

Vesicular Mole
Natural size

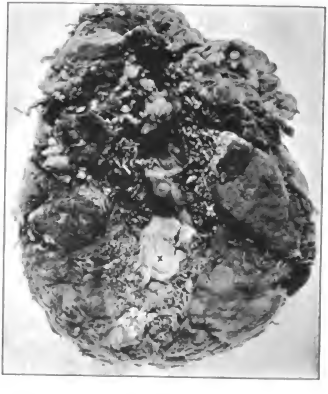

Fig. 6

Compact Vesicular Mole

Amniotic cavity
indicated by letter X

'VIRCHOW and FRANKEL say that if we have to do with syphilis of the father, which has left its mark on the ovum, we must expect to find it has exercised most of its influence on the chorionic villi.'

Endomentritis has been largely upheld as a predisposing cause, and in support of this is pointed out that multiple molar pregnancies have been known to occur in the same patient. ETHERIDGE mentions one case who developed the condition eleven times. Other pathological conditions of the uterus have also been suggested, such as fibroids, polypi, etc., but, so far, there has not been sufficient proof for these suppositions.

VEIT believes that structural changes in the maternal mucous membrane interfere with the proper circulation of the blood in the ovum, and thus the chorionic villi become oedematous, and vesicular mole is produced.

KIEFFER has advanced a similar theory, tracing the condition to proliferating arteritis, and this he attributes to the abuse of emmenagogues in early pregnancy. He was not able, however, to demonstrate his theory by any histological evidence, but the idea is of interest in view of one of the cases about to be recorded, where the patient had repeatedly taken pills to procure abortion.

BERRY HART, assuming the death of the foetus to be primary and not secondary, pointed out as a possibility (rather a remote one, perhaps) that, by the death of the foetus, the action of the thyroid is withdrawn, and so a myxomatous condition of the chorion results.

LUDWIG FRANKEL has shewn that there is a marked tendency to the development of vesicular mole in cases where ovarian cystoma already exists. This has been confirmed by other writers, among whom may be mentioned, MATVIEFF and SYKOFF, who recently exhibited at the Moscow Gynecological Society a gravid tube containing a typical vesicular mole. The adjacent ovary contained two corpora lutea in a state of cystic degeneration, and it is maintained by these observers that the cystic condition of the ovary was the 'undoubted cause' of the mole.

Although not prepared to accept such an unqualified statement, I fully believe that there is a close connexion between the two conditions. Out of the seven cases of which I have full notes, in two, the patients were operated on for cystic disease of the ovaries before the molar condition was recognized, and it is highly probable that ovarian cystic disease is present in a still greater proportion of cases where the symptoms have not called for operative interference, and so have remained unnoticed. It is interesting to note that in the history of the following case, the ovarian, and not the uterine condition was the one for which advice was sought.

The patient, aet. 29, was admitted complaining of a painful swelling in the side of eight months' duration. She had had six children, the last two years ago, no abortions. Menstruation was regular up to the onset of the present illness, when it ceased. During the following eight months of amenorrhoea, the pain in the side

increased greatly in intensity at what should have been a monthly period, and was accompanied by vomiting and a 'bearing down sensation.' There was no vaginal discharge. Abdominal section was performed on March 5th, 1901, and a large cyst, containing a thin clear fluid, was removed from the right side. At the operation it was noted that the uterus was wrinkled on its peritoneal surface, and equal in size to a four-and-a-half months' pregnancy.

On March 24, after a week's slight preliminary bleeding the patient expelled a vesicular mole (see Fig. 6, Plate IX).

That the existence of ovarian cystic disease is an important factor in the causation of vesicular mole has still to be proved, but as the two conditions have many points in common, it is probable that their occurrence indicates something more than a coincidence. Until, however, a greater number of cases are investigated with special reference to this point, the question must remain undecided.

Another fact, which must not be overlooked, is that a neurotic temperament frequently obtains in persons who are the subjects of this disease, and a history is often given in these cases of mental worry and distress, leading to much depression and general weakness. This may be regarded as an important predisposing factor.

As a primary cause, the circulation of a toxine in the maternal blood has been suggested, developed during gestation, and determining by its irritative qualities, degeneration of the chorionic villi. This, we believe, comes nearer the truth than any of the foregoing theories, but it is, in the present state of our knowledge, impossible to definitely prove it.

Frequency. The approximate frequency has been estimated at 1 in 20,000 pregnancies, but as will be readily understood, the estimate is liable to wide variations, owing to the difficulty in collecting such statistics, and the fact that many cases occurring in the early months of pregnancy are diagnosed as carneous moles and recorded merely as abortions.

Symptoms. These, stated briefly, are enlargement of the uterus, haemorrhage or amenorrhoea, a watery blood-stained discharge, occasionally containing vesicles, and the usual symptoms indicative of pregnancy.

In all the cases I have collected, the only constant symptom was enlargement of the uterus, but it is important to note that enlargement beyond what is expected from the period of gestation does not by any means occur in all cases, as so many writers of the text books would have us infer.

Out of seven cases, in one only was the uterus larger than the corresponding period of pregnancy; in all the others, the uterus was considerably smaller. In two cases, where the period of pregnancy was eight months, the uterus only corresponded in size to a four-and-a-half months' pregnancy; in another, a nine-and-a-half months' history to a six months' uterus, etc. This latter case is of special interest, as it represents one of the few cases in which a molar pregnancy lasted beyond the full term.

The patient, aet. 32, had had one child previously, but no abortions. The present pregnancy dated from September 26, 1892, the full term being due on July 3, 1893. On May 6 haemorrhage occurred, and the uterus was noted to be only at the level of the umbilicus. After a few weeks' rest in bed the bleeding ceased, but from July 12 to 18, a fortnight beyond the full term, bleeding was resumed, the uterus still remaining the same size. Labour was induced by Dr. Briggs, who placed in utero, four gum elastic bougies, and at the end of seven-and-a-half hours a large hydatidiform mole was expelled entire, the bougies not having penetrated any portion of it.

In one case only did the uterine enlargement correspond at all closely with the period of pregnancy, and there the history was of four-and-a-half months, and the uterus judged at the time of abdominal section to be four months.

Therefore, in describing the physical characters of the uterus in this condition, it is necessary to divide the cases into three classes.

1. Those in which the uterus is larger than the period of pregnancy would lead one to expect.

2. Those in which the uterus corresponds to the period of pregnancy, and

3. Those in which the uterus is smaller than the period of gestation.

The enlarged uterus is, as a rule, soft, fluctuating, and elastic, ballottement cannot be obtained, and in many cases marked tenderness is complained of. In some instances, however, the uterus is irregular on the surface and peculiarly hard and dense.

This was specially noted in the following case, where the density of the uterus gave rise to considerable trouble during the abdominal section, performed for the removal of ovarian cysts.

E.G., aet. 29, two children, no abortions.

Admitted complaining of severe vomiting and bleeding from the vagina. The last menstruation occurred about the middle of January, and towards the middle of April haemorrhage set in accompanied by severe vomiting. This persisted on and off up to the time of admission to hospital on May 28.

On examination, per vaginam, cystic disease of both ovaries was made out, the uterus being about the size of a four months' pregnancy.

On May 30, abdominal section was performed, multilocular cysts of both ovaries removed. The uterus, although corresponding in size to a four months' pregnancy, reached to the level of the umbilicus, being pushed up from behind by the cystic ovaries. Hardness of the uterus was noted at the time, as it caused much difficulty during the ligaturing of the pedicles.

On May 31 clots were passed per vaginam, and, labour pains setting in a few hours later, a large vesicular mole was passed early on June 1, accompanied by much haemorrhage and blood clot.

The next most constant symptom is haemorrhage. Although it is usual to get a history of amenorrhoea in the early months, sooner or later haemorrhage almost invariably occurs. In five out of every seven cases haemorrhage was a serious symptom. The blood loss may be slight and occur only from time to time, or it may be so severe as to endanger the patient's life, in which case rapid dilatation and evacuation of the mole are indicated.

A thin watery discharge, tinged with blood, is present in a small percentage of cases, but, when it is present, is a most valuable and characteristic symptom. When accompanied by the discharge of molar vesicles, the diagnosis is complete, but as this happens very rarely, too much stress should not be laid on its occurrence.

In two of my cases, however, a watery discharge was noted, and in one of these, vesicles were passed.

The history is as follows :—

Mrs. W., aet. 26, married four years, two children.

After the second child was born the patient suffered from subinvolution and endometritis. The last period ceased on December 7, 1900, and on April 17, 1901, a vaginal discharge was noted, at first watery, but later tinged with blood. It was, however, on account of headache, insomnia, palpitation (neurotic symptoms), and vomiting that advice was sought.

On examination, the fundus uteri was found two inches below the umbilicus, uterine souffle was made out, but no foetal heart sounds were heard. Per vaginam, there was glandular erosion of the cervix, the os was patulous and admitted the finger, on withdrawing the latter, a vesicle was found on it, and, almost immediately, two or three more vesicles were passed. The following day, after profuse haemorrhage, the complete molar mass was found lying in the vagina (see Fig. 7, Plate X). This was easily removed, and the patient made a good recovery. This is the only case I have to record in which the uterus was bigger than the period of pregnancy to which it corresponded.

Pathology

As shewn by the accompanying photographs, the vesicular mole is composed of a mass of grape-like vesicles springing from the chorion. These cysts are formed by the degeneration of successive parts of a villus, the unaffected portion forming the stalk of the next vesicle. They vary greatly in size even in the same specimen, small vesicles are seen springing from the larger ones, they possess no regularity or arrangement, and are attached by delicate filamentous stalks to each other and finally to the chorionic membrane. The whole mass may be enclosed in a thin covering of decidua, or the vesicles may be found to be lying almost free, the only traces of decidua being found at the upper end, which is usually

the more compact. In only one of my cases was there an amniotic sac (see Fig. 6, Plate IX), and there the mole was unusually compact, only small vesicles being present. There was, however, no trace of a foetus in any of the specimens.

The mole may be any size from two or three up to nine or ten inches in length, the size as previously indicated not having any definite relation to the length of the pregnancy.

The fluid contained in the vesicles is thin, clear, and watery, having a slightly reddish tinge, of low specific gravity, neutral in reaction, containing mucin and derived albumins, and debris of highly refracting granules which do not stain with osmic acid.

The mucin, which is a glycoproteid, is insoluble in water, and does not dialyze, it has, however, a marked capacity for taking up water, and this is easily demonstrated, for when a mole is placed in that medium, the cysts swell up and may become quite twice their original size. It has been said by some observers that vesicular moles do not contain mucin, but in each case I have examined, the chemical tests for mucin have given a positive result.

The fluid found in these cysts is formed by the breaking down of the myxomatous tissue of the central core of the vesicle, and to appreciate fully the minute changes which occur in this condition, it is necessary to have a clear idea of the structure of the core of the normal villus.

Here the stroma consists of connective tissue cells, which vary greatly in size and shape. Some are large with polygonal outline, having a long wavy process from each angle. The body of the cell is rich in protoplasm, and contains a centrally placed nucleus, which stains well with haematoxylin. The processes intertwine with those of neighbouring cells, so forming a complete network. Other cells are smaller, and tend more to the round or ovoid shape; they possess a well-stained nucleus, with one or more nucleoli, and are seen scattered irregularly throughout the stroma. In a full term villus, the stroma often presents a finely fibrillated appearance, with comparatively few nuclei. This is most marked in the neighbourhood of the capillaries, where the fibrillae assume a concentric arrangement enclosing the blood vessel, the wall of which is only represented by a single layer of flattened endothelial cells. The capillaries may be so large as to occupy almost all the stroma, or there may be a number of small ones in the same villus. These contain foetal blood shewing nucleated red blood corpuscles during the early months of pregnancy, poikilocytes, microcytes, and many varieties of leucocytes.

In vesicular mole, the stroma presents a strikingly different aspect. There is a marked absence of nuclei, the main mass of the stroma being made up of a few myxomatous cells with very long branching processes. The cells are much elongated, their nuclei are rod shaped, oval, or round, according to the pressure or stretching to which they are subjected. Between the cells is seen the finely granular matrix

containing the mucin. In some sections these granules have attained considerable size, and, as they stain very deeply with all the basic dyes, they stand out in marked contrast to the rest of the stroma. Whether these granules actually consist of the mucin secreted by the myxomatous cells is not certain, but it is quite reasonable to suppose that they do, since they possess all the physical characteristics of that substance.

That part of the stroma immediately underlying the epithelial layer is the most cellular, the nuclei becoming more and more scarce as the centre of the vesicle is reached.

In a vesicle about 2 mm. in diameter, the myxomatous tissue stretches right across, forming a very loose and delicate network, but in the large vesicles the central core has completely disappeared, the space being occupied by a thin watery fluid.

One of the most striking features, however, is the entire absence of blood vessels; so far as can be made out, the entire foetal blood supply has been cut off. This fact is of great diagnostic value, especially in those cases where small portions of retained placenta are removed by the curette, and the diagnosis of the condition rests on microscopical evidence. That such retained masses do sometimes take on a myxomatous degeneration with proliferation of the epithelium is beyond doubt, as two or three examples have come under my own observation.

The epithelium overlying the degenerated stroma also shews changes which are of great interest and importance. The most obvious of these is the marked proliferation of the cells, which in some specimens is so extreme as to suggest an adenomatous condition.

Both the syncytium and the zellschicht take part in the proliferation, sometimes one being more in evidence than the other, but in most cases the former presents the most striking appearances. The protoplasm of the syncytium stains even more deeply than in a normal villus, and possess nuclei of large size, coarsely granular and of very irregular shape. As in a normal villus, wherever the syncytium is in contact with the maternal blood, the nuclei shew the typical, elongated, and flattened appearance, and if the nuclei are two or even three layers deep, the innermost are always seen to be more oval and round as they approach the zellschicht. But as we have already seen, both syncytium and zellschicht are remains of the foetal trophoblast, therefore the proliferation which forms so striking a feature of this condition is not to be regarded as due to the increase of two different kinds of cells, but to one variety of epithelium which, according to its position on the villus, is seen to be flattened, plasmodial, oval, or cubical.

This proliferation of the epithelium takes place principally on the external surface of the villus, where it forms large heaped-up masses of cells. These may be cut off and form islands which appear to lie free in the blood spaces, while others grow out into long finger-like processes which may branch and give off

Fig. 7
Mole with large Vesicles.

Fig. 8
Wall of Vesicle.

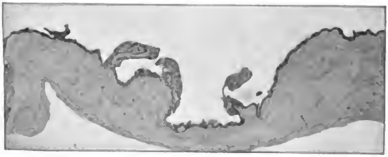

Fig. 9
Wall of Vesicle with Proliferation of the Syncytium.

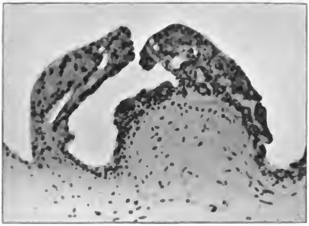

Fig. 10
Wall of Vesicle with Proliferation of Syncytium.
Higher magnification.

buds. These buds are somewhat typical in appearance. The nuclei are seen to be all massed together in the centre, forming what appears under the low power to be the dense deeply-stained nucleus of a giant cell. But on closer examination it is seen to be composed of a number of coarsely granular nuclei of very irregular shape, crowded together, and fitting into one another.

In other places the epithelial masses present a more reticulated appearance. Large oval spaces are seen between the cells, suggesting an exaggeration of the vacuolation process which takes place normally in the plasmodial cells of the trophoblast. According to Peters, two forms of degeneration occur physiologically in the trophoblast, namely, vacuolation and condensation, and it appears to me that in vesicular degeneration of the chorion, these two processes are carried on in a marked degree, giving rise to the network of syncytial cells on the one hand, and to buds of the same tissue on the other (see Figs. 8, 9, and 10, Plate X).

Almost more striking, however, than this external proliferation is that which I have remarked in many of my sections, namely, an invasion of the stroma of the villus by the epithelium. This is a point of great interest, shewing as it does the power of the foetal epithelium to penetrate into mesoblastic tissue, thereby causing its destruction. In places the ingrowths of epithelium give the appearance of a transverse section of a tubular gland, lying embedded in the stroma, completely cut off from the outer investing layers. The inner layer of this glandular looking structure is formed of deeply stained flattened nuclei, arranged round the lumen, while outside these, cubical and cylindrical cells are seen, having a definite cell outline and possessing oval nuclei.

These are not seen in all sections of vesicular mole, but when they are present they are a sign of great importance as indicating undue activity of the epithelium.

It has been said by some observers that it is possible from the microscopical appearances in vesicular mole to foretell the occurrence of chorion carcinoma. Whether this be so or not has yet to be proved, but it is interesting to note that in the one case of vesicular mole I have to record, which afterwards developed this condition, the sections did present some unusual features.

To the naked eye, the vesicles were of small size and surrounded by large masses of blood clot. Microscopically the stroma of the vesicles was seen to be completely disorganized, nothing remained of the myxomatous tissue, its place being taken by a homogeneous structureless network which stained a dark blue with haematoxylin. The outer covering, however, shewed great activity. Like the appearances described above, the nuclei were greatly increased, but the protoplasm of the syncytium appeared shrunken and very granular, as though undergoing some degenerative change.

Budding of the syncytium was very marked, but differed from that usually seen, in that the protoplasm formed a thin shell-like enveloping membrane, inside of

which were crowded dense masses of deeply stained nuclei. In that part of the syncytium which was in contact with the maternal blood, a curious fringe-like appearance was seen, and, although I have noted this in several other cases, it was specially well marked here. The fringes, which are best seen with an oil immersion lens, resemble cilia very closely, and it is reasonable to suppose that they are provided to help on the stream of blood through large spaces, where of necessity the circulation must be very slow, and also, perhaps, to present a larger surface for the absorption of nutrient material for the growth of the vesicle. As the whole structure has no blood supply of its own, it is dependent entirely on the maternal blood for its nourishment.

Another striking feature of this case was the enormous number of leucocytes which were present in the blood, bathing the vesicles. In places, the number of white corpuscles exceeded even that of the red, and suggested, very forcibly, the idea that some infective process was going on. Many varieties of white cells were seen, lymphocytes, large mononuclear and multinuclear leucocytes being specially numerous in those regions when the blood was in actual contact with a vesicle.

It would appear from this that the vesicles themselves were the source of irritation, the blood reacting to the stimulus and pouring out a dense leucocytic exudation, or it may be that the blood having been infected reacts again on the tissues of the mole, causing it to take on a malignant growth.

Whether this be so or not, one is convinced, after the study of a large number of sections of vesicular mole, that a very real and intimate connexion exists between vesicular mole and the condition presently to be described, namely, chorion carcinoma. Vesicular mole has been described by Frankel as a 'chorio-epithelioma benignum, and if the remains of a mole undergo malignant development there occurs a chorio-epithelioma malignum.' Other observers have described the condition as a cystic new formation, but the advocates of this view are few.

Virchow believed it to be a true hypertrophy of the villi with myxomatous degeneration of the connective tissue of the stroma.

Marchand, whose researches on this subject are of great value, lays emphasis on the proliferation of the syncytium and Langhans' cells, and regards the swelling of the vesicle as a dropsical condition produced mechanically and not a myxomatous degeneration.

PART III. CHORION CARCINOMA

This condition, under the name of 'Sarcoma Deciduo Cellulare,' was first described by SANGER, in 1888, as a 'malignant deciduoma-forming metastasis.'

In 1890, PFEIFFER independently published notes of a similar case, and proposed for it the name of 'Deciduoma Malignum.' Since that time considerable attention has been paid to the subject, especially by continental observers, and the literature is already somewhat extensive.

It seems doubtful, however, if all the cases published under this heading have been genuine cases of what is understood by the term 'Chorion Carcinoma;' and since there has been so much difference of opinion expressed as to its pathology, even by eminent workers, we are inclined to believe that three classes of cases have been described.

1. A true carcinoma developing from remains of a vesicular mole or retained chorionic elements.

2. A sarcoma of the uterus developed during and influenced by pregnancy, and

3. Ordinary forms of uterine carcinoma (EDEN).

As will be seen later, in studying the pathology of this disease, we regard the name 'Deciduoma Malignum' as misleading and incorrect, there being no evidence that decidual cells ever take on a malignant growth, and from what has already been seen, it is obvious that the syncytial cells for which they have been mistaken, bear only a very superficial resemblance to those found in the decidua.

The disease is a fatal one, death occurring in some cases as early as one month after the termination of the pregnancy. It is characterized by the presence of a uterine tumour, which grows with great rapidity, and is almost invariably accompanied by haemorrhages and an offensive discharge. It tends to infiltrate the wall of the uterus, even to perforation, and spreads locally by means of metastasis, which occur along the venous channels.

Etiology of the Disease

Age. Chorion Carcinoma may occur at any age during the child-bearing period, but is strictly limited to that period. Cases have been recorded as early as 15 and as late as 53, but the largest number seem to have occurred at that period when the maternal functions are in the greatest state of activity, *i.e.*, between 20 and 35.

But more important than the age factor is the nature of the pregnancy immediately preceding the development of the disease.

Quoting from CULLEN's *Cancer of the Uterus*, we find that—

One case followed a tubal pregnancy.

One case followed a miscarriage at the sixth month.

Twelve cases followed an abortion.

Seventeen cases followed a labour at term.

Twenty cases followed the discharge of a hydatidiform mole.

'In those cases in which abortion had occurred, two of the women had previously expelled hydatidiform moles. Thus in twenty-two (43·3 per cent.) of the cases there was a history of the expulsion of a vesicular mole some time prior to the appearance of the disease.'

Other writers give even a higher percentage than this, and, from my own observations, I believe it is probable that in all cases, whether preceded by a mole or not, changes take place in the epithelium of portions of retained placenta, which are the starting point of the disease, exactly similar to those which occur in vesicular mole. As previously mentioned, I have, in two or three cases, found evidences of such changes in pieces of retained placenta which have been removed by the curette.

It has been objected by EDEN that there is no evidence that pieces of placenta go on growing after the death of the foetus, but when it is remembered that the villi of the early ovum, and also in vesicular mole, are dependent for their nutrition on the maternal blood in which they lie, and not on the foetal supply, it is easy to understand how the chorionic tissue can go on growing long after the death and absorption of the foetus.

As an important predisposing cause, the general condition of the patient must be considered. In a large number of the cases recorded there have been disturbances of the general health during the preceding period of pregnancy. One symptom has been specially noted, viz., pernicious vomiting. This, in many cases, has occurred in the early months and led to premature emptying of the uterus. Other cases have developed neurotic tendencies—apprehensions as to the result of the pregnancy, persistent headaches, perverted mental conditions, and in one case maniacal attacks are said to have occurred.

In those cases where the disease is preceded by the expulsion of a vesicular mole, the patients are invariably anaemic and emaciated from frequent blood loss, and interference with the digestive functions.

SYMPTOMS

These, stated briefly, are sudden and severe attacks of haemorrhage, occurring within a few days or weeks of the termination of the pregnancy, rapid emaciation, profound anaemia, an intermittently high temperature, and all the other accompaniments of septic intoxication.

The symptoms usually set in shortly after parturition, though in a few cases, a brief respite occurs. On examination, the uterus is found to be enlarged, tender, and of a doughy softened consistence. Per vaginum, the os is patulous, but otherwise the cervix is apparently healthy. Small nodules may be present in the wall of the vagina, if metastasis has occurred. On passing the finger into the uterus, the whole or part of the interior is found converted into a raised boggy mass, resembling placenta to the touch. In places, deep excavations may be felt, and at these points the extreme thinness of the muscular wall is noted. Rupture of the uterus may occur, death of the patient following rapidly on the accident. In one of the cases about to be described, the muscular wall of the uterus measured only 1 mm. in thickness, the tumour being easily visible from the outside, shining through the attenuated muscular tissue.

Although curettage of the uterus should never be performed in this condition, in those cases where it has been done, it has always been accompanied by excessive haemorrhage, the blood gushing out in a most alarming manner. This is regarded by some as a point of diagnostic value, but it would be well if, in doubtful cases of this kind, the examining finger were used for the removal of pieces of tissue required for microscopical investigation, as haemorrhage once started, is extremely difficult to arrest, owing to the large blood spaces present in the tumour, and the inability of the thinned-out muscular wall to contract.

For permission to investigate the following case, I am indebted to the kindness of Professor Briggs.

The history of the case is as follows :—

Mrs. B., aet. 43, ten children, youngest aet. 14. No abortions. Menstruation normal. The last two periods ceased on May 9 and June 20, 1900. For some time previously, the patient, who was of a neurotic temperament, had been subject to much mental strain, caused by domestic troubles, and her general health had become much impaired. Towards the end of August, when she considered herself about three months' pregnant, she began taking pills to procure abortion, and these she continued to take in September and October.

On September 7, uterine haemorrhage set in, and the patient became anaemic, her general condition being by this time very low.

On October 12, she was seen by Dr. Briggs, who described the uterus as feeling harder than the normally pregnant uterus, and diagnosed a vesicular mole. The uterus was larger in size than would be expected from a pregnancy dating from June 20, but smaller than one dating from May 9. Owing to the weak state of the patient, no attempt was made to rapidly empty the uterus. Three gum elastic bougies were placed in utero at nine p.m., and the mole was naturally expelled on the evening of the next day.

After the expulsion of the mole, the patient continued to bleed, the amount lost being small. Symptoms of septicaemia now appeared, and the temperature rose and fell irregularly, sometimes rising as high as 105°, with occasional rigors.

On November 12, four weeks after the expulsion of the mole, the uterus was explored with the finger, and a large ulcerated patch with indurated edges was made out on the posterior wall near the fundus (see Fig. 11, Plate XI). Extreme softening of the wall was noted, and it was feared that perforation was imminent.

Operation was delayed for a day or two in hopes that the patient's condition might improve somewhat, but as she steadily became worse, the uterus was removed per vaginam on November 28. The patient survived the operation only eight days.

Unfortunately, a *post-mortem* examination could not be obtained, so that it is not known if metastatic deposits were present.

The uterus was somewhat enlarged, the body appearing more spherical than normally. On laying it open a large necrotic haemorrhagic area was seen on the posterior aspect of the fundus, extending over the orifices of both fallopian tubes.

The growth presented a number of small rounded elevations, several of which were dark red in colour and extremely soft to the touch. Although depressed towards the centre of the tumour, the edges were raised from the surrounding mucous membrane, bulging forward into the cavity of the uterus, the growth also penetrating into the muscular wall, almost as far as the serous coat. The mucous membrane lining the remainder of the cavity appeared normal, and the cervical canal shewed nothing abnormal in its entire extent.

The condition found in the vesicular mole expelled by this patient has been already described, and it will be remembered that the sections shewed intense leuycocytic exudation round the vesicles. This indicates that the septic infection had already occurred before the mole was expelled, and as there was marked proliferation of the syncytium, with a great increase in the amount of syncytial buds lying free in the blood spaces, it is probable that metastasis, as well as the malignant infiltration of the muscular wall, had also begun.

But the greatest interest attaches to the uterine tumour, in that it shews on microscopical examination two vesicles deeply imbedded in the muscular coat, and from them the syncytium is seen budding off and making its way into the venous channels. Looking at a section, beginning at the internal surface, we find in places remnants of the mucous membrane, which is very thin and atrophied. The glands are small and lined by a single layer of rather cubical epithelium which in places has fallen out, but they present no other features of importance. It is, however, worthy of notice that the inter-glandular stroma appears to have gone back to its normal condition. No trace of decidual cells is to be seen, only spindle-shaped cells with small round or oval nuclei are present.

In most of the sections, the mucous membrane has disappeared, its place being taken by blood clot, partially organized in places and shewing much leucocytic exudation, in others more recent haemorrhages are seen. Here and there, adherent to the

surface of this, are seen the remains of villi, most of which have lost their epithelium, the myxomatous core being all that is left. Close to, but separated by the above-mentioned semi-organized blood clot, are masses of granular protoplasm, containing a number of oval nuclei. These are deeply stained and shew great variety in their size and shapes. They are seen penetrating into the muscular coat, at times, as a long strand making its way inwards towards the blood spaces by which the muscular wall is intersected; at others, breaking off into small buds or even isolated cells. These wandering cells appear to possess great powers of penetration, as they are met with in all parts of the section. They stain deeply, and so stand out prominently from the rest of the tissue, the nuclei being especially rich in chromatin.

About midway between the mucous and serous coats two hydatidiform vesicles are seen, surrounded in the greater part of their extent by a haemorrhagic area. The stroma of the vesicle is composed of myxomatous tissue, towards the centre it is becoming thinned, and spaces are seen which probably during life were filled with fluid. There is no sign of any overgrowth in the stroma, and it does not penetrate at any point the investing epithelial layers. It is to these latter that the special interest of the section attaches (see Figs. 12 and 13, Plate XII).

The epithelium is present, covering the entire surface of the vesicle. Both syncytium and Langhans' zellschicht are well seen, and both are in a state of active proliferation. The cells lying in immediate contact with the stroma are almost cubical in shape, though in places much irregularity exists, and they are arranged in rows, sometimes as many as six layers deep. Next to these, and in contact with the surrounding blood, is the syncytium. This is in a more active state of proliferation than the zellschicht, large masses of protoplasm are seen budding off, sometimes in long club-shaped strands, at others, in small rounded masses.

Vacuolation of the syncytium is very marked here, giving the appearance of a network of protoplasm, with delicately-curved nuclei, following the outline of the reticulum. Tracing these to the periphery, it is seen that, wherever the syncytium comes into contact with the muscular wall, syncytial cells have penetrated far and wide, and plasmodial masses may be observed, having a direct connexion with the vesicle on the one hand, and maternal blood spaces, deeply embedded in the muscular wall, on the other.

At a short distance from the vesicle just described, but separated from it by muscular tissue, a large clump of cells is seen lying free in a venous channel. They correspond in every particular with the syncytium covering the vesicle, and are evidently derived from that layer. As the lumen of the vessel is almost entirely occupied by this mass of cells, it is probable that multiplication is going on *in situ.* Near to the serous coat more villi are seen, also associated with large haemorrhagic areas, some of which shew recent blood and dense leucocytic exudation. Many of the blood spaces shew invasion by the syncytium, so that from the microscopical

evidence it is highly probable that metastatic deposits had occurred, although their existence could not be verified.

Gebhardt has recorded a case in which an hydatid villus was enclosed in the tumour, while Apfelstadt and Aschoff record a remarkable instance of 'malignant disease of the uterus following the spontaneous evacuation of a vesicular mole; both the primary tumour and a metastasis in the labium majus were found to consist of a cluster of vesicles, having the structure typical of the vesicular or hydatidiform mole.'

Although many observers have suggested that chorion carcinoma is developed in the above manner, observation of the process under the microscope has only been possible in very rare cases, and it is admitted by all, that it is only by the careful study of the conditions existing in the vesicular mole, when it is known to precede this disease, that a true solution of the pathology of chorion carcinoma can be hoped for.

The second case I have to describe was under the care of Dr. Nathan Raw, of Liverpool, to whom I am indebted for the clinical notes and permission to investigate the case.

The patient was a married woman, aet. 39, who was admitted on January 18, 1902, to Mill Road Infirmary, Liverpool, complaining of profuse haemorrhage and offensive discharge from vagina, which had occurred on and off ever since a miscarriage three months previously.

No information could be obtained about the pregnancy, but the foetus was thought to be of about the seventh month.

On admission, the patient's temperature was 99°, but from that time it gradually rose, and on January 25, preceded by a rigor, it reached 105°. The injection of 20 c.c. of antistreptococcic serum reduced the temperature to normal, and the patient remained fairly well till February 13, when a severe flooding occurred. On the 15th there was another rigor and rise of temperature to 104°.

February 17. The haemorrhage and offensive discharge still continuing, chloroform was given and the uterus curetted. During the operation the haemorrhage was most profuse, blood gushing out in a most alarming manner. This was arrested by packing; the patient, who was now extremely anaemic, remaining free from bleeding until March 2, when there was another rigor, followed by a sharp rise to 105·2°.

March 11. Rigor and rise of temperature to 106·2°, accompanied by the passage of a large clot.

March 12. Another rigor and rise of temperature to 105°.

March 20. 'Since the last injection of antistreptococcic serum, the temperature has dropped nearly to normal. She is still very pale, and perspires every night. There is now a soft blowing systolic murmur heard at the apex, and evidently

mitral. This seems to be infective, and has just developed within the last two weeks.'

From this date up to the beginning of June, the patient remained much in the same condition ; there were frequent rises of temperature, on two occasions as high as 106°, preceded in each instance by a severe rigor. On June 8 severe pain was complained of in the lower abdomen, and, on the following day, death ensued.

During the last few days of the patient's life, the uterus was noticed to increase in size, somewhat rapidly.

The cervix appeared normal, but nodular swellings were felt bimanually at the fundus. The extremely low condition of the patient prevented any operative interference.

The *post-mortem* examination revealed an enlarged uterus ; the body had assumed an almost spherical shape and shining through the thin, almost transparent uterine wall ; dark red haemorrhagic-looking tumours were seen.

The appendages, although slightly congested, appeared otherwise normal.

On laying open the uterus, the body is seen to consist merely of a thin membrane of pale muscular tissue, measuring 1 mm. in thickness, enclosing a bleeding fungating mass, which corresponds in general outline to the original wall of the uterus. This gives the impression that the malignant growth had begun at the mucous membrane, and by some inherent phagocytic action had completely replaced the original tissue. On the inner surface of the growth, forming an almost complete lining to the cavity, is a greenish-yellow slough. Filling up the cavity, but adherent only at one point, is a large projecting tumour mass, resembling in appearance and consistence a placental polyp. This also is covered in places by a purulent exudation.

The recent appearances are reproduced in the accompanying drawing (see Fig. 14, Plate XI).

In the lungs numerous secondary deposits were seen. These varied greatly in size, a few being as large as a walnut, but by far the greatest number only about the size of a green pea. They felt hard to the touch, and on section were seen to correspond exactly with the primary tumour. A fibrous looking capsule enclosed each nodule, so that they appeared sharply cut off from the surrounding lung tissue. The smaller deposits were distinctly paler than the larger ones, which were more haemorrhagic, and, therefore, appeared darker in colour.

An ill-defined area of pneumonia surrounded the tumours, and it is remarkable that these did not give rise to any symptoms during life. The bronchi appeared normal.

On examination of the heart, a few brittle vegetations were found on the curtains of the mitral valve.

The rest of the organs, beyond absence of fat and an undue pallor resulting from the profound anaemia, shewed nothing of interest.

Pieces of the primary tumour including the muscular wall, and of the secondary deposits in the lung, were hardened in Plimmer's solution, and a number of sections cut. Examining the primary tumour, no trace of the uterine mucosa was to be found, the internal surface being occupied by a mass of necrotic tissue, consisting of fibrin, a few spindle cells, blood corpuscles, dense masses of leucocytes, and a number of badly stained cocci. Owing to the difficulty in demonstrating these latter satisfactorily, it is impossible to say definitely if they were streptococci, though in several instances, the organisms were seen in small chains, and as the tissue had been put into hardening fluid before it was sent to me, I was unable to make cultures from it.

Passing outwards, we find that this superficial area of necrosis is not sharply demarcated from the adjacent tumour tissue, but becomes gradually merged into it. In this neighbourhood, the remains of cells can be clearly made out, from most of which the nuclei have disappeared, although a faint outline can be traced in some instances.

A number of small broken-up protoplasmic masses are seen, staining badly, almost entirely devoid of nuclei and of those granules which are so characteristic of the growing syncytium. It is to be specially noted that neither here, nor in any other part of the section, are decidual cells present. The uterine glands have entirely disappeared, and no trace is found in any section of the superficial epithelium.

Passing further outwards, we come on the true tumour tissue. This is seen to be in a state of active growth. As seen under the low power, the most characteristic points to be noted are the irregular masses of cells forming a coarsely reticular meshwork, enclosing large haemorrhagic areas. On closer examination of the blood in these spaces, it is found that, in the larger areas, disorganization of the corpuscles is going on and fine threads of fibrin are seen traversing the coagulum.

In the smaller spaces, the blood appears more recent. The red corpuscles have retained their shape and taken on a pink stain with eosin. The white cells are seen to be greatly in excess, their nuclei have stained well and shew many varieties, the large multipartite and mononuclear predominating. It is specially noted that the blood here is not enclosed in blood vessels, or even in spaces lined by endothelium, but lies free, in direct contact with the tumour cells now to be described.

On examining, even with the low power, it is at once apparent that two varieties of cells are present ; one kind made up of irregular bands, oval or circular masses of protoplasm containing numerous deeply staining nuclei ; the other smaller, oval cells, in some places massed together in such profusion that no cell wall is visible, having smaller and more transparent nuclei. These cells alone, together with the blood spaces already described, make up the entire mass of the tumour. There is no connective tissue stroma between the cells, and no intercellular structure of any kind has been observed.

On closer examination, the protoplasmic bands are seen to correspond in every respect to the syncytium previously described. The ground substance is coarsely granular, stains well with eosin, and contains numerous vacuoles. On the surface, which is in contact with the blood, especially in those areas where the haemorrhage is most recent, the peculiar ciliated or fringed appearance is again noted, and adherent to these fringes a red corpuscle may here and there be seen.

It will be remembered that this appearance is very characteristic of the syncytium covering the vesicles in hydatidiform mole, and, in my opinion, is a point of great importance as demonstrating the intimate connexion between these two conditions.

Lying in this protoplasmic substance, large numbers of nuclei are seen. The shape of these varies according to the position they occupy. Where the syncytium (as we shall now speak of it) is drawn out into long wavy strands, the nuclei are long, spindle, or rod-shaped, where a sharp curve is made, the nuclei are correspondingly curved or crescentic. In many places, however, the syncytium appears spread out into a flattened mass, with branching processes, or by cross section of a strand, oval and circular discs are seen, bearing a close resemblance to giant cells. Here the nuclei sometimes assume a large size, or there may be one or two very large oval nuclei, accompanied by several smaller ones. The oval or circular masses often contain a group of nuclei closely packed together, and, as they are always found lying free in a blood space, they resemble in every minute particular, the syncytial buds which are seen not only in sections of hydatidiform mole, but also in normal villi, and even more markedly in the early stages of the development of the chorion.

The nuclei of these giant cells are rich in chromatin, and, therefore, stain deeply with haematoxylin and with the aniline dyes, they shew numerous granules and frequently a well-defined nucleolus.

Separated from the blood spaces by a thin layer of syncytium are seen the other variety of cells. These are so densely packed, that in very few places any definite cell can be made out. Where this is visible the cell appears oval or round, its protoplasm is not quite so granular as that of the syncytium, and does not stain as deeply.

The nuclei are pale and transparent, and contain one or more nucleoli in almost all cases. When a number of granules are present, they are arranged at the periphery of the nucleus, leaving the centre clear and transparent. Nuclear vacuoles are also seen here and there. These cells are scarcely ever seen isolated, but are massed together in dense columns, and it is most interesting to observe that these cells do not come into actual contact with the blood, but are separated from it on all sides by a layer of syncytium (see Figs. 15 and 16, Plates XII and XI).

As the tumour approaches the muscular wall, there are evidences that this is the growing edge. The haemorrhages are more recent, and the cells take on the

stain more accurately. There is no sharp line of demarcation between the healthy tissues and the invading cells, though it would appear from the uniform thickness of the muscular wall that the process of infiltration was proceeding with uniform rapidity. Masses of cells are seen making their way in between the muscular fibres, and at this point the involvement of the maternal blood vessels is seen. As shewn in the illustration, the cells approach the wall, penetrate into the lumen of the blood vessel, thence to be carried by the blood stream to some distant part of the body.

Sections of the secondary deposits in the lung shew precisely the same structures as those found in the uterine tumour, so that it is unnecessary to recapitulate the description. It is, however, noticed that the nodule is more necrotic, the central part being composed almost entirely of blood clot and disintegrating tumour masses, so that the typical syncytial strands and the oval cells are seen most abundantly at the periphery, where they form a kind of investing membrane. The cells may be traced penetrating into the haemorrhagic area, either as fine strands, or as broader flattened masses.

The protoplasm of the syncytium is more vacuolated than that of the primary tissue, and it appears in places as though these vacuoles had been formed by the dropping out or absorption of a nucleus. Intense leucocytic exudation is here also a marked feature.

In the immediate neighbourhood of the nodules, the lung tissue is seen to be in a state of congestion. The alveoli are choked with exudation, and the walls are thickened and inflamed. A bronchus close by also shews inflammatory changes, with secretion of mucus.

From the consideration of these two cases, which, although differing somewhat as regards the clinical course, agree in that the pathological elements found in each are identical, it is argued that this condition is one of true carcinoma, arising in the chorionic epithelium, and parasitic on the maternal organism.

As shewn in the earlier part of this paper, the epithelium covering a villus is a strictly foetal structure, being the remains of the trophoblast, and further, that according to their position relative to the villus and the maternal blood, the cells assume either a flattened plasmodial form, the syncytium, or retain their primary cubical or polyhedral shape, the so-called zellschicht of Langhans.

Taking this view, the difficulty experienced by so many observers, in assigning a separate origin for the two varieties of cells found in this condition, vanishes. It is evident that, as in the early villus, the pressure of the blood, and possibly some chemical action of its plasma, can convert the polygonal cells of the trophoblast into a flattened condensed mass of syncytium, so, later in its history, the blood laden with some toxic element, can so act on the chorionic epithelium undergoing malignant proliferation, as to cause condensation and formation of syncytial masses in precisely the same way.

FIG. 11
Uterus with Chorion Carcinoma
(Mrs. B.)

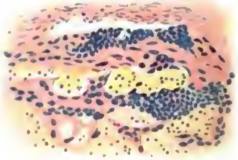

FIG. 16
Section of Chorion Carcinoma shewing
involvement of maternal blood space

FIG. 14
Uterus laid open from behind
(Chorion Carcinoma)

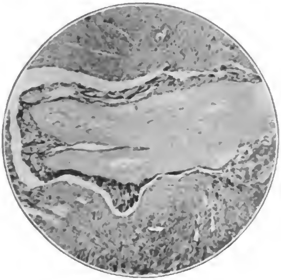

Fig. 12
Vesicle embedded in Muscular Wall of Uterus.

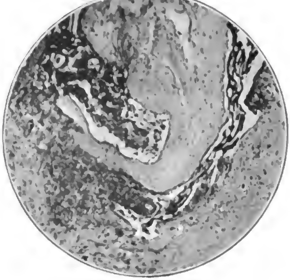

Fig. 13
Vesicle embedded in Muscular Wall of Uterus.

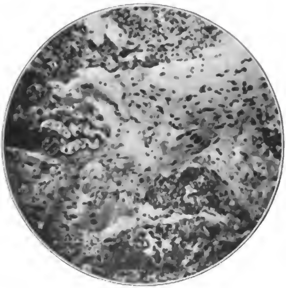

Fig. 15
Section of Chorion Carcinoma.

It would appear from all the sections I have examined, that it is essential, both for the normal growth and malignant proliferation of the syncytium, that it be in immediate contact with maternal blood.

As shewn by PETERS in his specimen, the trophoblast was intersected by blood spaces, communicating with the maternal sinuses, and on following the syncytium through all the stages of its existence, it is seen to be surrounded by maternal blood, not only normally in the placenta, but also in vesicular mole. Naturally, one would expect to find the same conditions existing in the malignant degeneration of the chorion, and they do, for in every place where syncytium is found, blood is also found in the immediate vicinity, not only in the primary focus, but also most markedly in the secondary deposits.

The presence of the smaller more discrete cells of LANGHAMS appears to be by no means constant. Whether this is due to the uneven rate of growth or to the variations in the general blood pressure is uncertain, but it is interesting to note that in a number of the recorded cases, only one variety of cell is mentioned as composing the growth, and that invariably the syncytium.

This is another argument in favour of both cells having a common origin in the trophoblast, seeing that, at any stage of its existence, the latter may be converted into syncytial masses.

Through the kindness of Dr. HUME, of Baltimore, I have had the privilege of studying sections of WHITRIDGE WILLIAMS' specimen, and have found it to agree, both in the primary and secondary growths, with the appearances described in my second case.

The first case supplies its own proof, since the connexion of the degenerated villi with the malignant tissue is actually observed under the microscope, the two varieties of cells being seen *in situ* not only attached to the villus, but penetrating far and wide into the muscular coat and blood vessels of the uterus.

It has been allowed by all those who have hitherto disbelieved in the foetal origin of this disease that, if it can be shewn that the elements comprising the tumour are chorionic, the required proof will be found in the study of vesicular mole. It is on these lines that I have tried to trace the life history of these cells, from the trophoblast of the ovum in its earliest stages to its later development, as seen in a fully-formed villus and in the benign and malignant growths found in vesicular mole and chorion carcinoma respectively.

It has been objected by many English and American writers, that this condition should be regarded as a rapidly-growing sarcoma, developed during pregnancy, and shewing unusual changes due to the genetic influence of that state.

While quite prepared to admit that such sarcomata may exist, and shew characteristic changes due to the influence of pregnancy, it has still to be proved

that the many cases described as 'Deciduoma Malignum' really do belong to that class, especially when the microscopic findings, as shewn by photo-micrographs, etc., shew structures which are obviously of foetal origin.

One observer alone suggested that the tumour arises from foetal connective tissue.

Gottschalk, in recording a case where the disease developed after an abortion at the sixth week, described the condition as a sarcoma of the stroma of chorionic villi. The plasmodial layers, although seen to be in an active state of proliferation, budding not only into surrounding tissues but also into neighbouring blood vessels, he regards as unaltered by disease, and maintains that the malignant change affects the connective tissue of the stroma only.

In the light of our present knowledge, this explanation falls to the ground, since a malignant growth of the stroma could not give rise to proliferation and metastasis of the syncytium.

Marchand believes this condition to be a carcinoma of structures arising from the foetal ectoderm, and Whitridge Williams, although leaving the origin of the polymorphous cells an open question, regards the syncytial masses found in his specimen as arising from chorionic villi, and, therefore, of foetal origin.

In conclusion, I may add that Virchow made an invaluable discovery when he found that every tumour had a physiological prototype, a statement that may well be applied to the disease we have described, since chorionic elements are found normally during the period of pregnancy in close connexion with the maternal tissue, and have been shewn by recent observers to have the power of reaching distant parts of the body, without producing any untoward results.

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ON A CHARACTERISTIC ORGANISM ASSOCIATED WITH CANCER OF THE BREAST

ON A CHARACTERISTIC ORGANISM ASSOCIATED WITH CANCER OF THE BREAST

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IN this paper an account is given of certain researches, part of a series conducted during the last five years on the subject of carcinoma. These researches have been confined to one clinical type of cancer, that affecting the female breast. Although it is possible that the cause of cancer is universally one and the same, arguments to the contrary can be adduced, and research is simplified by narrowing its limits. The following are the subdivisions of the paper :—

1. Previously reported research.
2. The method of examining the growths.
3. The morphology of the organism isolated.
4. The histology of the growths examined and the culture results obtained from each.
5. Commentary.

I. PREVIOUSLY REPORTED RESEARCH

In a paper read before the Royal Society on December 14, 1899, an account was given of certain organisms isolated from carcinomata of the breast and uterus. They were classed as blastomycetes. They were isolated on glucose agar ; other media were employed, but growth occurred on the glucose agar medium alone. Subcultures were obtained on wort agar and potato, and in wort bouillon and neutral bouillon, and their characteristics described. A special method of staining the organism in the tissues was described, and its morphological characters summarized as follows :—' Fresh specimens from cultures are spherical, from four to ten microns in diameter, and in most cases take an aniline chromatin stain diffusely. There is, however, a great variety in the distribution of the chromatin ; it is sometimes aggregated to one pole, sometimes divided up at different parts of the cell, and in other cases it is represented by a few isolated granules. The capsule is delicate. Multiplication in cultures takes place by budding.

' In the primary growths produced by intraperitoneal inoculation of the organism, the latter is also in most cases spherical, possesses a delicate capsule,

and multiplies by budding. Two peculiarities are, however, to be seen : firstly, in many cases delicate processes connect adjacent specimens of the organism; secondly, the capsule is often thickened and forms a kind of 'halo' round the central deeply-staining body of the cell.

'In the nodules in lungs, liver, spleen, and kidneys, which are secondary to the growths in the peritoneum, in addition to the forms already described, spore-bearing forms are found. In these the capsule of the cell is much thickened, the chromatin of the cells breaks up irregularly, and portions are allowed to escape through dehiscences in the capsule. There is no regularity in the process, no simultaneous division of the cell contents into a definite number of spores, and no simultaneous shedding of the same. The spores are without capsule when shed, and are irregular in contour. They stain deeply with chromatin stains, and are finely granular.'

II. THE METHOD OF EXAMINING THE GROWTHS

The tumours, the examination of which is described in this paper, are seven in number. In regard to Nos. 1 and 6, I was not present at the operation, but the tumour was in my hands within an hour and within two hours, respectively, after removal, and was conveyed from the operating theatre to the laboratory in sterile coverings. With regard to the other tumours, I either removed them myself or was present at the operation. Immediately after removal, each was wrapped in sterile gauze, outside of which was placed one or more sterile towels. I then brought it to the laboratory, and, after sterilizing my hands and instruments, I removed the coverings so as to expose the deep surface of the mass ; the skin surface remained downwards in contact with the sterile coverings. With a knife or spatula heated in the flame the surface was then seared, and incisions made into the tumour. Small portions were then removed and immersed in the fluid media or rubbed over the surface of the solid media. Scrapings of the cut surfaces were also taken with the platinum loop and inseminated in the same manner.

With regard to the examination of the animals inoculated : immediately after being killed or as soon as possible after death, the abdomen was shaved and scrubbed for some minutes with lysol (4 per cent.) The peritoneal cavity was then opened, and either the whole or parts of the organs affected removed and placed in sterile petri dishes. The surfaces of the organs were then seared with the heated spatula, and incisions made into the lesions. From the latter, pieces or scrapings were removed and inseminated. All manipulations were carried out as rapidly as possible. The tubes were in all cases incubated at 37° C.

I have employed the following media :—nutrient broth, nutrient agar, milk, glucose broth (4 per cent.), glucose agar (1 per cent.). lactose agar (1 per cent.), ascitic fluid, ascitic fluid broth, ascitic fluid agar, ascitic fluid glucose broth, human blood

serum, human serum with ·2 per cent. glucose, and a medium prepared from carcinoma material with 2 per cent. peptone added.

In three of the four cases in which positive results were obtained, I used glucose broth, glucose agar, and milk alone. In one case, the organism developed in ordinary nutrient broth. These are the only media in which growth occurred.

III. THE MORPHOLOGY OF THE ORGANISM ISOLATED.

The following description is based upon the study of the organism (*a*) in the primary cultures obtained from the tumours, and in subcultures from these, (*b*) in the cultures obtained from the lesions produced in animals, and in subcultures from these.

In the chapter which follows this, I shall describe in what way, and by the use of what methods the various types of the organism have been found associated with the several growths and animal lesions.

For the sake of clearness in description, the forms under which the organism occurs in cultures are here divided into two main types, A and B. These types are directly related, and are merely two phases in the life history of the organism. Their separation is artificial, and based only on morphological grounds. It is also for the sake of clearness and simplicity in description that I first take type A, a form of the organism which does not appear in the cultures isolated in the first instance from the tumours, and secondly, describe type B, which is the form which occurs in these cultures.

The following is the order of description :—

1. The morphology of type A.
2. The method of reproduction of type A.
3. The morphology and development of type B.
4. The macroscopic appearances of the cultures.

1. The Morphology of Type A

The following description is derived entirely from fresh undried specimens, unmounted. Cultures on solid media are prepared by placing a drop of water on the slide, giving this a slight tinge with the stain employed, and placing in the drop a particle of the culture. In the case of fluid cultures, a loopful is taken and lightly coloured. A cover glass is then placed on the drop, and the specimen examined immediately. Dried preparations give in most cases what have been ascertained to be quite unnatural appearances, and to some of these reference will be made later.

(*a*) *Shape.* Living specimens are spherical, oval, and lemon-shaped ; in old cultures are found crenated forms and other irregular types. Actively-growing forms in young cultures are all spherical.

(*b*) *Size.* The size varies between 4μ and 8μ in diameter. In certain cultures there are specimens larger than this (circa 10μ) ; such forms occur in old cultures and in cultures which are degenerate and showing no tendency to develop. Many of these large forms are ruptured, and are in reality but empty capsules.

The maximum and minimum diameters given, 4μ and 8μ, represent the limits of the size of the type in healthy recent cultures.

(*c*) *Structure.* There are two chief varieties of type A present in all cultures in varying proportions.

The following descriptions are of cultures all stained with methylene blue* (Plate XIII, Figs. 1 and 2) :—

Variety 1. The organism possesses a capsule which is very delicate in most specimens, but in older cultures it is denser and possesses a double contour. Within this is a delicate cell network and nucleus, both of which are with difficulty made out. The whole cell either remains colourless or takes a diffuse pale rose colour, and neither cell network nor nucleus are chromatophylous.

In the majority of specimens are one or more highly refractile granules. They usually take no stain, but occasionally take a dense blue.

These granules in young cells are scattered in different parts of the interior, but in other cells are collected into a spherical or oval mass, situated in the centre or slightly eccentrically.

In these cells, with central mass of granules, no cell network outside this mass can be seen, nor a nucleus distinct from it. Vacuoles are seen in many of the cells, especially in older cultures ; occasionally these contain a refractile granule, either motionless or exhibiting active 'dancing' movements.

Variety 2. This variety shews a definite capsule of varying density. Within this is a mass of finely granular protoplasm which takes an intense blue colour. This intensely coloured protoplasm may either completely fill the interior of the cell or may present itself as a spherical mass, with an uncoloured ring of apparently structureless protoplasm between it and the capsule. At or about the centre of this granular chromatophylous protoplasm a nucleus can be distinguished.

As already stated, both these varieties are to be seen in all cultures.

In young glucose agar cultures variety 1 is almost exclusively seen, specimens of variety 2 are few in number. In young glucose broth cultures the two varieties occur about equally in number. In glucose broth cultures, four days old and onward, variety 2 is the more common ; in such cultures there is always a sediment, and in this sediment growth variety 2 predominates to a greater extent than in the suspended part of the culture.

If dried coverslip preparations of the organism are made and stained, certain peculiar appearances are obtained. In the process of drying, the organisms

* Methylene Blue, 4 grms., Sod. Bicarb., 2 grms., Water, 400 c.c.

run together in clumps, and are faceted against each other. The protoplasm of the interior shrinks away from the capsule and presents various irregularities of shape.

2. Method of Reproduction of Type A

Both of the above described varieties exhibit a method of multiplication by budding (Plate XIII, Fig. 1). In the case of variety 1, the method is as follows:—The bud appears first as a slight bulging of the capsule; this gradually becomes more distinct; the interior of this projection appears to be homogeneous in structure. As it develops it assumes a spherical contour, and its capsule is derived from the unbroken capsule of the parent. When near to full formation an intracellular network is to be distinguished, and when the parent cell possesses scattered refractile granules, one or more usually appear in the bud. I have not been able to distinguish any process of karyokinesis of the parent cell nucleus. Separation of the bud goes on gradually; it may be delayed until a series of three or more is formed. When young cultures are kept at the room temperature these chains are not formed, but single daughter cells are produced and separate off; if such cultures are incubated at 37° C. the process is active, and a single parent cell may exhibit two or more buds, forming simultaneously in different stages towards separation. In the description of variety 1, it was stated that in older cultures many of the cells shewed an aggregation of refractile and deep blue stained granules at the centre; this type of cell rarely shows bud development, but in buds produced from these cells there is a gradual division of this nucleus-like granular mass which progresses with the formation of the bud; a portion eventually becomes completely separated off and passes into the bud. In the case of variety 2, the bud produced may possess either characters similar to those of the parent, or the characteristics of variety 1. In the latter event, the process of formation is similar to that just described, except that the capsule of the bud is not simply derived by distension from the complete capsule of the parent; the outer layers of the capsule of the parent cell are ruptured, and the bud is projected out with a covering derived only from the inner layer or layers. When this variety 2 produces a bud resembling itself the process is essentially similar to the budding of variety 1. The interior of the bud is granular, and takes a deep blue colour, and it may derive its capsule from the whole or from part only of the parent cell capsule; in many cases the latter is so delicate that it can be distinguished only with difficulty.

2a. On a certain Process observed in Glucose Broth Cultures of Type A

When a fresh glucose broth culture of A is made by inseminating a particle of glucose agar culture, after twenty-four hours' incubation there are present forms which exhibit the following process:—At a single point in the capsule there is to be seen a minute dehiscence or micropyle, and through this the granular contents of the cell are being protruded. The cells which shew this process possess a well-marked

and sometimes dense capsule, and the interior structure is of that form, in which there is a central or somewhat eccentric mass of aggregated granules (Plate XIII, Fig. 3).

The protruded material is either in the form of discrete spherical granules or of very delicate fibrillar material, in which granules are entangled. The granules thus discharged may be either deeply stained blue, when methylene blue is used, or may be uncoloured and highly refractile. The micropyle is distinctly seen as a minute channel, on the one hand, continuous with the central granular interior of the cell, and on the other, with the exterior through a stoma on the surface. The cells exhibiting this process are, many of them, pear-shaped, being somewhat distorted in the direction in which the extrusion is taking place ; the outline of the interior and the extrusion may be compared to the core and the stalk of the pear. This distortion is not, however, common to all discharging forms. The continuity of the extruded granular material and the granular material in the centre of the cell, through the micropylon, is definitely seen.

If the same culture is examined at the end of forty-eight hours' incubation, it is characterized by the large number of cells which shew this process of extrusion (Plate XIII, Fig 4). The extruded material is, in most cases, still attached to the cell by its stalk, but there are also free in the culture small masses of spherical granules. These masses of small spherical granules have the appearance of minute cocci of varying size, connected together by intercellular fibrillar material. I use the term coccus in a descriptive sense only.

After seventy-two hours' incubation (Plate XIII, Fig. 4), there is present in the culture an increased amount of this small coccus-like growth ; the individual cells of this are spherical, and take a deep blue colouration with methylene blue. The large cells from which extrusion has taken place are much vacuolated and without granules. The free granules occur singly, in pairs, and in groups, and shew a larger average size than those in the earlier cultures.

I have examined many of these glucose broth cultures after more prolonged incubation, but, except in two instances, have been unable to determine any further development of this growth of small spherical granules. When such cultures are plated on glucose agar colonies of A develop after three or more days, according to the age of the culture, but, with the exception of the two occasions which I have just mentioned, no colonies of any other type. On these two occasions there multiplied in the glucose broth cultures of A an organism of a type much smaller than the A specimens, spherical and oval in shape, and from 5μ to 3μ in diameter ; these organisms were abundant in the cultures, and when plated on glucose agar developed as separate colonies after twenty-four hours' incubation at 37° C.

Example 1. Glucose broth culture inoculated May 16 ; plated June 1 on glucose agar ; colonies of the small type alone developed ; microscopically in the glucose broth both the small type and the A type were seen, the former abundant.

Example 2. Glucose broth culture inoculated May 31; plated June 22 on glucose agar; colonies of both small type and of the A type developed. Microscopically the glucose broth culture shows the presence of both types, the small type being abundant.

These are the only two examples, as I have already stated, of the separation of a small cell type of organism from cultures of type A. On the other hand, I have had many examples of the development of a similar small cell type when cultures of A are inoculated into animals.

I take one example of this from my previous paper:—

1. Guinea-pig inoculated intraperitoneally with organism of A type. *Post-mortem* lesions found in peritoneum, spleen, liver, lungs, and kidney. In these lesions groups of organisms are seen, which consist of A forms surrounded by a small type of organism in large numbers. Such a group is shown in the photograph (Fig. 2, Plate XIV).

The following examples are from the present series of observations:—

2. Guinea-pig inoculated, April 9, 1900, with pure culture of A. Killed April 24. From the lesions a small cell type of organism isolated in pure culture.

3. Guinea-pig inoculated, February 6, 1903, with pure culture of A. Killed February 17. From the lesions a small cell type of organism isolated in pure culture.

4. Guinea-pig inoculated, February 10, 1903, with glucose broth culture of A. Killed March 6. From the lesions, colonies both of A and of a small cell type separated.

5. Guinea-pig inoculated, June 4, 1903, with glucose broth culture of A. Killed June 12. From the lesions glucose broth cultures show a few A forms, but the greater amount of the growth consists of a small cell type. These cultures were plated, and colonies of the latter alone developed.

6. Guinea-pig inoculated, June 4, 1903. Killed June 16. From the lesions glucose broth cultures were recovered, which showed, microscopically, both A forms and a small cell type form. Glucose agar plates show numerous colonies of the latter after twenty-four hours' incubation at 37° C. After forty-eight hours a few A colonies also developed.

This small cell type of organism, thus isolated in two instances from cultures of A in vitro, and in many instances from the lesions produced by the inoculation of A, shows constant morphological characteristics, and a mode of further development uniform in all cases.

It is an organism identical with this in type and characteristics that I have isolated from the tumours examined.

The circumstances attending its development in cultures directly derived from the carcinomatous tumours will be given in detail in the following chapter; although, as will be seen, in one of the cases A forms were also present in small numbers in the primary cultures obtained from the tumours, this small cell type, which I shall in future call type B, is the type to be associated with these primary cultures, and type A usually appears later as a result of the inoculation of type B into animals.

I have said that I have isolated the small cell type under three different sets of circumstances:—

1. From cultures of A in vitro.
2. From animal lesions produced by the inoculation of A.
3. Direct from the carcinomatous growths.

The characters of the growth being similar under all these circumstances, the following description of type B is common:—

3. The Morphology and Development of Type B

Cultures examined microscopically in the fresh state show an organism for the most part spherical in form; there are, however, present, especially in primary cultures from the growths, other shapes, *i.e.*, oval and club-shaped forms. The size of the spherical forms varies from that of an extremely minute granule to an organism 3μ to 4μ in diameter. The cultures all show marked viscidity, and the organisms are seen microscopically in masses and clumps, with a large amount of intercellular material connecting them. When stained with methylene blue these masses have a very characteristic appearance; the dark blue organisms stand out in a field of intercellular fibrillar material, which takes a reddish to red purple colour. The oval and club-shaped forms occur indiscriminately among the spherical forms in the clumps; they are more marked in some than in other cases. If cultures of B procured from either of the abovementioned sources are studied in subcultures, it is seen that the average size of the organisms tends to become slightly smaller and more uniform, and the oval and club-shaped forms disappear.

When inoculated into animals, type B usually undergoes important modification. Cultures which have not been carried through more than two or three subcultures in vitro are pathogenic to guinea-pigs and dogs, and produce in these definite lesions; cultures which have passed through several generations lose pathogenic activity, and this loss of pathogenicity occurs especially early in the case of the cultures derived directly from the carcinomatous tumours. In rabbits I have not observed the development of any definite lesions after intraperitoneal inoculation of B, but when cultures are inoculated intravenously the organisms can be again recovered from the blood in a form which shows modification from the type injected.

B has, therefore, been studied as recovered from the lesions produced in guinea-pigs and dogs, and from the blood of rabbits inoculated intravenously. The

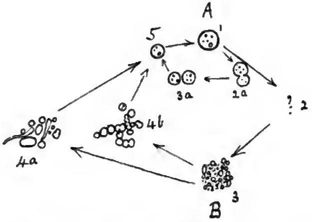
changes which it has shown have varied according to the circumstances attending its inoculation, and the following examples are illustrative :—

1. Guinea-pig inoculated intraperitoneally with B culture from a carcinoma on February 6, 1903 ; cultures recovered February 16, showing an organism of larger average size than that inoculated and with a greater variety of shape (Fig. 4, Plate XIV), the shapes noted being spherical, oval, oblate-spheroid, club-shaped, and forms of great length, with club-shaped extremities ; some of the large spherical forms are as large as the specimens seen in cultures of A.

2. Guinea-pig inoculated, April 6, 1903, with B culture from carcinoma. Killed May 4. Organism recovered of larger average size than that inoculated, and shews a mycelium-life development. This culture was inoculated intravenously into a rabbit on June 2, and recovered from the blood on June 5 by introducing ·5 c.c. of blood in 2 c.c. glucose broth. In these cultures the mycelial growth was marked, the individual organisms forming the mycelium were of large average size, and the largest shewed the characteristics of type A. A was not, however, isolated as a separate growth from the cultures.

3. Guinea-pig inoculated, November 27, 1902, with culture from carcinoma of type B. Killed January 1, 1903. From the lesions there were recovered cultures, which consisted in part of type B of larger average size than in the injected culture, but also abundant growth of typical type A forms. From these cultures both B colonies and A colonies were isolated on glucose agar plates (Fig. 2, Plate XIV).

The changes which have been observed in the two types A and B, and the relations between the two types, in so far as they have been traced, may be diagrammatically shewn.



1. A type. 2. (?) Intermediate form from which B develops. 3. B type. 4A and 4B. B as modified in animals. 5. Form resembling A, seen in 4 and 4A cultures. 2A. Budding form. 3A. Bud separated, which resembles 5.

In regard to this diagram there are two stages shewn which require comment, 2 and 4 (A and B). To take the latter first, it has not been shewn that B in its

evolution towards A, must necessarily pass through the forms which are shewn as 4A and 4B; it may be that the mycelium-like growth and the diverse forms of 4A are rather involution-like forms of B than an essential stage in its development. With regard to stage 2, I have been unable up to the present, to decide it with certainty. In the description of type A, a process has been given in detail, which is to be observed in glucose broth cultures, which results in the extrusion of minute granular forms from the A forms. As has been already stated, this granule growth has not been seen in these cultures to develop to more than a very limited extent, and in the great majority of cases no colonies develop, except colonies of A, when such cultures are plated. The two exceptions have also been related. Under the circumstances, I am not able to state that this process of dehiscence and extrusion is a real phase in the life history of the organism, and the question must be left for further study.

In my previous paper I described a process of sporulation to which I have referred in Chapter I of this paper. In the series of observations which I am now reporting I have not seen this process with the same clearness, but the process of dehiscence and granule extrusion resembles it, though it does not reproduce all its characteristics. Therefore, although there is evidence pointing to the fact that stage two is represented by a spore-producing form, yet for the present this is inconclusive. In the last place, I wish to refer back to the statement above, that the cultures of type B which obtained from the tumours are identical in character with those of type B obtained from the lesions produced in animals by the inoculation of type A. This statement is based on the facts (1) that the naked eye appearances of the cultures on the ordinary media are alike; (2) that microscopically they possess the following characters in common:—The specimens are very variable in size in single cultures; the groups show marked polymorphism and many different shapes; this polymorphism is most marked in first cultures from the tumours or animal lesions, and in subcultures there is a species of reversion to a spherical organism which varies chiefly in the size of the specimens; mycelium-like growth develops in each case under certain circumstances.

4. Macroscopic Appearance of the Cultures

Type A

Glucose agar. In twenty-four hours abundant white growth along the needle track on sloped media; becomes heaped up along this track, and develops a yellowish and later a yellowish-brown colour.

Glucose gelatine. Slow, but abundant, development of dense white growth along the needle track and on the surface of stab cultures. No gas formation in the medium.

Potato. Yellowish-white abundant growth, develops as a broad heaped-up streak with crimped edges.

Nutrient agar. Scanty white growth in stroke cultures, becoming slightly yellow in old cultures.

Nutrient broth. Slow development of growth, which first appears after three days as a slight sediment.

Glucose broth. Abundant growth after twenty-four hours' incubation, partly as sediment and partly suspended. Older cultures, three days, shew plentiful sediment growth, with supernatant fluid almost clear.

Type B

Glucose agar. Grows as a whitish-yellow translucent streak along needle track on sloped medium, with discrete spherical colonies alongside streak in some cases. Culture is extremely viscid, and, in some cases, can be peeled off the surface like a membrane.

Glucose gelatine. In stroke cultures grows as a pearly-white streak ; in stab cultures a feather-like growth along the needle track. No gas formation. Culture viscid.

Potato. Whitish-yellow ribbon-like growth ; very viscid.

Glucose broth. In most cases grows readily ; in twenty-four hours, at 37° C., causes a general cloudiness in the medium ; after three days there is also slight sediment growth.

Both types of organism were cultivated in a 2 per cent. solution of glucose in ordinary tap-water. Type A developed readily, type B scantily. In neither case did alcoholic fermentation take place.

IV. THE HISTOLOGY OF THE GROWTHS EXAMINED AND THE CULTURE RESULTS OBTAINED FROM EACH

1. On April 5, 1900, a tumour from the breast of a young woman was kindly sent to me by Mr. F. T. Paul. Sections showed a highly cellular carcinomatous growth ; the cells of large size in masses and columns ; there was very little fibrous over-growth between the cell masses.

The tumour was examined in the manner described in Chapter II ; the media employed were ordinary nutrient broth and nutrient agar. After three days' incubation, slight sedimental growth was seen in two of the nutrient broth tubes. This consisted of organisms with the following characters : groups and masses of spherical forms with a fine intercellular faintly staining fibrillar material between the individual specimens ; the latter varied in size from that of an exceedingly minute granule to a cell with a diameter of about 4μ. In dried coverslip preparations the organisms stained readily with methyl violet ; the smaller specimens deeply, the larger less intensely.

Subcultures were taken from these in glucose broth and nutrient broth and on to nutrient agar, glucose agar, blood serum, and nutrient gelatine ; no growth

developed on the solid media, but further cultures were obtained in the nutrient broth, and to a less extent in the glucose broth. These subcultures were of the same type as the primary cultures, except that the size of the organisms did not vary to such an extent; the proportion of the minute to the largest specimens in the original cultures was about 1 : 15; in the subcultures about 1 : 8.

Only one series of subcultures of the growth was obtained, and the organism thus rapidly lost vitality in the media employed.

Inoculation experiments were made into guinea-pigs and dogs. The histology of the lesions thus produced will form the subject of a later communication, and I confine myself here to the facts which have to do with the life of the organism.

On April 9, a guinea-pig was inoculated intraperitoneally with primary broth culture, and on May 11, a second was similarly inoculated with subculture from this. Both animals were killed, the first on May 1, the second on June 8; in both cases nodules were present, distributed generally throughout the peritoneal cavity, and in the spleen, liver, lungs, bronchial, and mesenteric glands. From these nodules, endeavours were made to obtain cultures, and from those in the animal killed on May 1, cultures grew on glucose agar, but none were obtained from the second animal. The cultures obtained from the first animal were identical in appearance with that injected.

On further injection of these cultures, no lesions resulted in guinea-pigs, and a similar negative result was obtained with all subcultures of the original culture, with the exception mentioned above.

The nodules on the peritoneal surfaces just described were broken up with pestle and mortar in normal saline solution, and injected through a series of animals, as shewn in the following table:—

Guinea-Pig 1
(Inoculated, April 9. Primary culture. Killed, May 1). Nodules.

Guinea-Pig 2
(Inoculated, May 11. Primary culture. Killed, June 8). Nodules.

Guinea-pig. Inoculated, May 1. with emulsion. Killed, May 28. Nodules.

Dog. Inoculated intraperitoneally, May 28. Killed, June 29. Nodules omentum. Spleen and liver.

Guinea-pig. Inoculated June 29. Emulsion. Killed, August 8. Nodules omentum. Liver, spleen.

Dog. Inoculated in mammary gland, August 8. Nodules in mamma and spleen.

Guinea-pig. Inoculated, May 1, with emulsion. Killed, June 7. Nodules.

Guinea-pig. Inoculated, June 7. Died, June 16. Nil.

Guinea-pig. Inoculated, June 7. Died, June 19. Nil.

Thus the nodules produced in the guinea-pig inoculated on April 9, 1900, and subsequently in another animal on May 11, were transplanted through four other animals in series. In each case, attempts were made to recover cultures from these nodules, but unsuccessfully.

Scrapings from the peritoneal nodules in guinea-pigs were examined fresh in hanging-drop preparations; there were seen in these, two types of cells which were distinguishable from the connective tissue and blood cells. The one type was of large size, an average of about 5μ with distinct capsule, and containing in the interior one or more refractile granules; the second type was a small spherical organism about the size of a minute coccus.

2. On November 24, 1902, I obtained a tumour from the breast of a pregnant woman, aged thirty-three; she was five months advanced in pregnancy, and the growth was of rapid development. The parenchyma of this carcinoma consisted of large cells with large oval nuclei, arranged as branching columns in a fibrous stroma; in the interstices of the stroma were numerous infiltrating round cells. In parts adjacent to the carcinomatous growth the breast shewed a condition of mastitis, the fibrous stroma being increased and infiltrated with round cells in large numbers, the ducts and alveoli were dilated. The media used in the examination of this tumour were milk and glucose broth; the method of examination was that described in Chapter II. Within forty-eight hours there was a general turbidity in most of the tubes of glucose broth; microscopic examination shewed this to consist of organisms of type B, as described in the preceding chapter, that is to say, spherical organisms of very varying size, in masses and groups, with a considerable amount of filamentous material connecting the individual specimens in the masses. Inoculations of these cultures were made into guinea-pigs as follows—five cultures being employed:—

Inoculation	Killed	Result
a. November 27 ...	December 30	Nil
b. November 27 ...	January 1	Lesions
b. December 8 (dog) ...	March 17	Lesions
c. November 27 ...	December 30	Nil
d. December 5 ...	January 5	Nil
e. December 5... ...	January 5	Lesions

Thus of five cultures two only produced lesions in guinea-pigs on intraperitoneal injection; the inoculation of the dog was made into the mamma.

From the three animals, cultures were obtained as follows :—

1. Guinea-pig. Culture types A and B.
2. Dog. Culture type B.
3. Guinea-pig. Culture type A.

The culture marked *b* in the table above was further injected into animals as follows :—

Animal	Inoculated	Killed	Result
1. Guinea-pig	January 26	February 4	Nil
2. do.	February 6	February 16	Lesions
3. do.	February 6	February 17	do.
4. do.	February 6	February 19	do.
5. do.	February 23	March 27	do.
6. Rabbit	February 23	April 27	Nil

From the animals, 2, 3, 4, and 5, in only one case were cultures recovered, viz., number 2. These presented certain peculiarities ; there were present organisms of type A, also in large numbers, rod-shaped, club-shaped, and ovoid forms (Fig. 4, Plate XIV), and thirdly, forms corresponding to type B. A second peculiarity of these cultures was that in subcultures the organisms belonging to type A disappeared, also the involution (?) forms and the growth consisted exclusively of organisms belonging to type B.

The inconstancy of the results obtained in the inoculations of the cultures in this case are remarkable. As seen in the second table, animals inoculated with the same culture, and even on the same day, gave contrary results. The only explanation for this inconstancy is the varying susceptibility of the animals used.

3. On March 4, 1903, I removed the left breast of a woman, aged forty-two. The whole breast was indurated. Above and to the outer side of the nipple was a nodule the size of a walnut, ill-defined. The right breast was also indurated and slightly tender. On microscopical examination the nodule referred to shewed a carcinomatous structure with branching columns of epithelial cells, the rest of the breast shewed marked hypertrophy of the interstitial fibrous tissue, with masses of inflammatory leucocytic infiltration. The investigation of the tumour was carried out in the manner described in Chapter II. In this case inoculations were made on to sloped glucose agar alone, by rubbing pieces of the tumour over the surface of the medium. Of ten tubes thus inoculated, after forty-eight hours' incubation at 37° C, growth was plentiful in two, scanty but present in the rest.

The growth in all tubes was of type B, with the variations of shape marked, the shapes noted were spherical, oval, club-shaped, and spindle-shaped ; the organism grew in clumps and masses, and in some of these club-shaped forms were arranged in a rosette-like manner.

The vitality of this organism in subcultures proved very limited, and glucose broth subcultures were obtained from one of the glucose agar cultures alone. These subcultures were injected into animals as follows :—

Guinea-pig subcutaneously	March 9 to March 27	Nil
Guinea-pig subcutaneously	March 9 to April 27	Nil
Guinea-pig intraperitoneally	March 10 to March 13	Lesions
Rabbit intraperitoneally	March 23 to April 28	Nil
Guinea-pig intraperitoneally	April 6 to May 4	Lesions

Thus of two guinea-pigs inoculated into the peritoneal cavity, one, inoculated with a culture five days old, died in three days ; the second, inoculated with a culture thirty-three days old, was killed twenty-eight days later.

On examination of the first, general peritonitis was found with yellowish-white nodules in the spleen and liver ; on examination of the second, similar yellowish-white nodules were found in liver and spleen, but no peritonitis. Cultures were recovered from both these animals ; those recovered from the first resembled the inoculated culture, but the club-shaped forms were more numerous, and, except that the average size was smaller, it was also similar to the cultures described on p. 175 recovered in case 2 from the peritoneum of a guinea-pig.

The cultures recovered from the second of the guinea-pigs was of type B as described.

In the case of the first animal, the cultures recovered were of extremely low vitality, and further propagation was not possible, while those from the second animal grew readily. It was, therefore, possible to follow certain morphological changes which took place in these subcultures. Those on glucose agar were of the usual B type, but those in glucose broth developed a mycelium-like growth of interlacing branches, the branches being composed of individual organisms of shaped ovoid, spherical and oblate, jointed together ; this mycelial growth is described in Chapter III, p. 175, and the separation off of spherical forms resembling type A is there noted.

4. On March 30, 1903, the patient above referred to as case 2, was again operated on for a recurrence of the growth. From the material removed, inoculations were made in the usual manner on glucose agar and into glucose broth. Growth

occurred in all tubes. The cultures were examined in the fresh state; they consisted in all cases of growth of type B. As in the previous cases the growth shewed (*a*) the typical variations in shape, (*b*) the typical variations in size; the variations in shape were club-shape, spherical, oval, lemon-shape; the variations in size ranged from an extremely minute spherical organism to club-shaped forms, $5\,\mu$ in length. The organism grew in clumps and masses. In the glucose broth cultures there were, however, organisms of type A, in small numbers; they exactly corresponded to variety 1 of type A, as described in Chapter III.

From these cultures no subcultures were obtained; primary cultures were injected into two guinea-pigs; one of these was killed by mistake three days after inoculation, when nothing abnormal was found; the other was inoculated intraperitoneally, April 6, and killed on May 13; nodules were found in the spleen, but the attempt to obtain cultures from these failed.

In addition to these four cases, three others were examined culturally in the usual manner; in one case, the cultures were contaminated with an organism apparently staphylococcus epidermidis albus; in the other two cases, no cultures were obtained.

V. COMMENTARY

In the four cases recounted the cultures isolated had the following characteristictics in common:—

1. The variations in size.
2. The variations in form.
3. The appearance during culture or inoculation of a certain characteristic form described as A.
4. The macroscopic appearance of the cultures.
5. The pathogenic effect on animals.

In case 1, the A forms were seen only in hanging-drop preparations from scrapings of the nodules; on the appearances seen in such scrapings not much reliance can be placed, and it is only by comparing drawings of the A forms observed in this case with the appearance of A isolated in cultures in the other cases that I was able to decide their presence with confidence. Many very peculiar forms are to be seen in such scrapings, and certain types of A are sometimes closely simulated by red blood-cells, leucocytes, fat globules, and nuclei of epithelial cells. The examination of scrapings from normal tissues, lymphatic glands, spleen, and breast tissue, has convinced me that deductions from such preparations in the case of carcinomata are exceedingly liable to fallacy.

Similarly, in the case of attempted culture by immersing pieces of carcinomatous tissue in fluid media, elements of the tissue become free and disseminated in the medium, and again may give rise to false deductions unless corrected by control

observations with pieces of normal tissue. I may instance one example: when blood is shed into a tube of glucose broth or ordinary broth, and an examination is made at the end of twenty-four hours, free spherical globules in large numbers are seen, which absorb aniline dyes and simulate organisms closely; red blood-cells are also seen, from which the interior protoplasm has diffused through the capsule and is adherent to the latter in the form of globules, taking a deep chromatin stain with methylene blue.

Case 2 is especially valuable in that typical A forms were found to develop in animals injected with B, while in one instance, the identical culture from which A forms developed in the animal, on subsequent injection as a subculture gave rise to what I have termed involution forms, the club-shaped and mycelium-like forms depicted in Fig. 4, Plate XIV.

Case 3 supplemented this observation by showing that the mycelium-like forms probably represented a stage in the development from B to A.

Case 4 shewed that although B was the type to be specially associated with primary cultures, yet A might also be found in these primary cultures.

Throughout the research the difficulties attending the culture of the organism have been illustrated, and also the manner in which the age of the cultures and the circumstances attending inoculation vary the results obtained by injection.

I do not propose to discuss the significance of the association of this organism with these cases of carcinoma mammae. The morphology indicates that it belongs to the vegetable kingdom and is related to the lower fungi. It is of great interest to read in a recent communication by ALESSANDRI* that he has isolated from two cases, one a fibrosarcoma of the forearm, the other a spindle-celled sarcoma of the stomach, cultures which, in the first stage, contained large spherical and oval forms with a double contour, and later, in association with these, small bodies in pairs and groups resembling sarcinae. The short description given of these cultures resembles in certain respects the account of the organism which I have given above, although these were non-pathogenic to animals, and the relation of the small forms to the large was not ascertained. The research reported in this paper agrees with, but amplifies considerably, that reported in my previous paper. In conclusion, I wish to express my indebtedness to my colleagues at the Northern Hospital, Mr. HARRISSON and Mr. MURRAY, for placing material at my disposal, and to Professor BOYCE, Professor Ross, and Professor MOORE for kind criticism and advice.

* *Centralb. f. Bakter*, Bd. xxxiii, No. 9, p. 686.

DESCRIPTION OF PLATES

PLATES XIII

Fig. 1. Culture of A in glucose Agar twenty-four hours old. Shews two chief varieties (1 and 2). Budding forms (3).

Fig. 2. Sub-varieties of A. Glucose broth three days old.

Fig. 3. A forms shewing first stage of extrusion. Note escape of fine granules through micropyla.

Fig. 4. A forms shewing further development of extrusion process. Note free spherical granules of larger size than in Fig. 3.

Fig. 5. A forms with B forms from glucose agar culture.

Fig. 6. Mycelium form. Note that free specimens are similar in type to A forms.

PLATE XIV

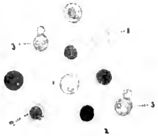
Fig. 1. Type B, from a culture isolated from case 2.

Fig. 2. Type A associated with Type B, in the lung of guinea-pig.

Fig. 3. Type A associated with Type B, from a lesion in a guinea-pig, produced by the inoculation of B.

Fig. 4. Type B, 'involution' forms.

I



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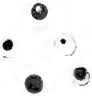

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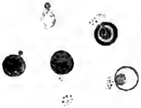

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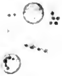
V



VI

PLATE XIV

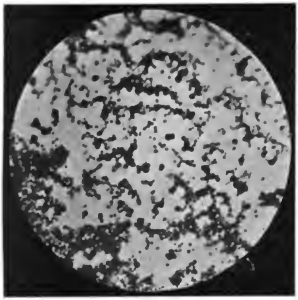


Fig. 1

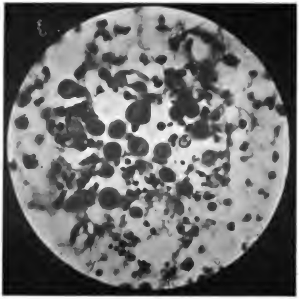


Fig. 2

Fig. 3

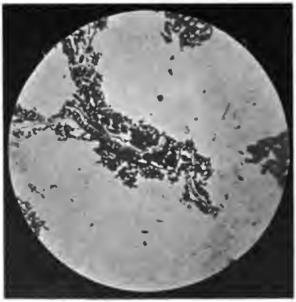


Fig. 4

'TICK FEVER' IN MAN

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'TICK FEVER' IN MAN

By CUTHBERT CHRISTY, M.B., C.M., EDIN.
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

SOON after reaching the Uganda Protectorate in July, 1902, I was informed by a native that in Toro (Western Provinces) there was an 'insect' whose bite caused serious illness. He described it as a spider, and said that it came out of the ground. Upon instituting a search at one of the camping places, I had brought to me specimens of the tick figured in the accompanying plate. I subsequently discovered that this tick, known to the natives as the *bibo*, was fairly common in Usoga, Uganda, Budu, German Central Africa, Toro, Unyoro, and at Waldelai on the Nile. It is most easily collected by searching the dust and straw on the floors of the huts erected for caravan porters, or the houses of the natives, though in these latter it is not so easily found when the floors are kept clean. Near Kampala the natives collected them for me around the bases of the verical supporters of the roof.

Their colour when alive is greenish-brown (see Plate XV). The largest specimens are about 8 mm. long by 6 or 7 mm. broad. On the dorsal surface of the eight-legged adults are from four to six coloured spots. The ticks are flat when unfed, and have several deep furrows on the back, but when distended with blood these furrows are less evident. They bite at night, and fall off when gorged. They are frequently carried long distances in mats or bedding, or in porters' loads which have been piled for safety in the rest-huts at night. Some specimens I collected at Fort Portal, in Toro, had been carried in bags of salt from Katwe at the north end of Lake Albert Edward, more than fifty miles away.

The natives of the Protectorate, particularly in Toro, dread this tick, and know well the symptoms occasionally following its bite. In describing these, they invariably go through a pantomime indicative of vomiting, with pain in the head and abdominal region. They say that the above symptoms may last for several weeks, but are never followed by fatal results. The great majority of natives are immune and suffer no ill effects from the bite, presumably having been immunized by previous bites.

I examined several cases of illness amongst my porters and others, in which the above symtoms were prominent, without finding anything peculiar in the blood. The men in each case blamed the *bibo*.

The worst case was one of my servants, a Swahili from Mombasa, where there is no *Filaria perstans*. Six days before being taken ill he had, contrary to his usual habit, slept in a native hut at a camp where the tick was very abundant. His main

symptoms were violent headache, fever, constant vomiting, pain in the abdomen, and some purging. For a time I almost gave up hopes of his recovery, but seven days after the onset he had nearly recovered his usual health. There was no enlargement of liver or spleen, or other physical signs that I could discover.

On several occasions during the months previous to his illness, I examined this man's blood without finding *Filariae*. During his attack, and for a week after it, the blood examinations were negative. Two months afterwards, however, I again examined his blood, and found a *Filaria perstans* embryo. Subsequently I found two more.

The distribution of the tick as I found it in Equatorial Africa, agrees to some extent with that of *Filaria perstans*, or at least with one of its sub-species ; and in a district near the north end of the Albert Edward Nyanza, where the *bibo* was abundant, I discovered that, not only was a larger percentage than usual of the natives infected with *Filaria*, but their blood in very many cases was so full of *perstans* embryos that I could count a hundred and fifty or two hundred on one slide.

From these facts, together with the apparently permanent immunity and other considerations, I have been lead to assume that the above-described symptoms, known amongst human diseases as 'tick fever,' a very different thing to the tick fever of cattle, are the result of a primary inoculation with *Filaria perstans*, by the tick known in Uganda as the *bibo*. In other words, I believe the tick here described acts as intermediary host for the *Filaria perstans*.

As inoculation and sectional experiments cannot for some time be completed, and as the occurrence of ticks harmful to man has not, I believe, hitherto been reported from Uganda, I have thought it best to publish this note without further delay. I shall go into the subject in detail in a forthcoming publication.

Mr. R. I. Pocock, *of the British Museum, has kindly furnished me with the following note upon the specimens which I submitted to him for determination :—*

The ticks above referred to by Dr. Christy are identical with those collected in Angola by Dr. Welwitsch, which Andrea Murray described in 1877 as *Argas moubata*, stating that the essential difference between this species and the earlier described Egyptian *Argas savignyi* is the presence of the pale integumental spots, independently observed by Dr. Christy, in the former—and well seen in a live specimen amongst those he has submitted to me. The specimens are also specifically identical with examples in the British Museum, collected by Dr. Dowson at Tete, on the Zambesi.

PLATE XV

The species is now known as *Ornithodorus moubata*. As I pointed out in 1900, it may be distinguished from *O. savignyi* by the invariable absence of the two pairs of eyes present in the latter. Hence it has recently, but quite unnecessarily, been renamed by NEUMANN, *O. Savignyi var. coeca*. NEUMANN records it from the Transvaal, etc., so it is evidently widely distributed throughout Central and Southern Africa. Dr. CHRISTY's discovery of the species in Uganda and his testimony as to its prevalence almost throughout the country comes as a surprise after the statement in JOHNSTON's 'Uganda Protectorate' that he did not remember 'having encountered or heard of that poisonous tick,' with which he was well acquainted on the Zambesi. It is an interesting fact that the testimony of Drs. LIVINGSTONE and WELWITSCH, Sir JOHN KIRK, Dr. DOWSON, Dr. DANIELS, and Sir HARRY JOHNSTON, with respect to the sickness which follows the bite of this tick, both in Angola and British Central Africa, has been independently confirmed by Dr. CHRISTY, who, at the time, did not know that the *bibo* of Uganda is identical with the *moubata*, *tampan*, and *carpato* of the Zambesi watershed. I am not aware that the allied species *O. Savignyi*, which has been recorded from Egypt, Nubia, Abyssinia, Somaliland, British East Africa, etc., shares the bad reputation of *O. moubata*. The geographical areas of the two species overlap. Hence, without the examination of actual specimens, it would be impossible to say which of the two species is involved in any given case of 'tick-fever.'

BLACKWATER FEVER

BLACKWATER FEVER

By J. W. W. STEPHENS, M.D., Cantab.
WALTER MYERS LECTURER IN TROPICAL MEDICINE, LIVERPOOL

I. THE DISTRIBUTION OF BLACKWATER FEVER

ONE of the commonest objections urged against the malarial origin of blackwater is that the distribution of blackwater and malaria is not identical. But it is equally true that the distribution of mild malaria and severe malaria is not identical, and the conclusion that blackwater is non-malarial is, for this reason alone, by no means justifiable. Thus, for instance, the mortality from malaria in the few still remaining foci of Northern Europe is not on a par with that of the Roman Campagna, nor again is the severity of malaria in Northern Italy and Austria at all comparable with that in Southern Italy and Sicily. Thus, Schaudinn[1] says, 'in Istria (on the Adriatic) the "tropica" always is very mild. There are no records of deaths, which are frequent in Italy.' The aspect of the 'virulence' of a particular species of parasite has, in this argument, been overlooked, and we cannot speak vaguely of malaria as a whole. The factors which determine 'virulence' of a parasite are almost entirely unknown. Those factors which constitute the climate of a country are, in all probability, the determining factors also of 'virulence,' whether this results from altered 'virulence' of the parasite itself or from altered blood conditions of the patient or from both.

In this paper, then, I propose to consider the distribution of blackwater fever, with a view to shewing that, so far as the data allow, there *is* a close correspondence between that of blackwater fever and of severe malaria. I do not propose to argue therefrom that blackwater fever is malarial, because the proof of this statement is not merely a matter of argument, but is based on definite facts. To these I shall return in a subsequent article. We may first consider the United States as here the data, more especially those regarding malaria, are sufficiently complete for a comparison to be made.

According to Hirsch,[2] the first records of blackwater fever in the United States date from 1866, in Texas and Louisiana, and subsequently in Mississipi, Arkansas, North Carolina. Davidson[3] gives the same list, Georgia, however, instead of Arkansas. Weber[4] mentions Alabama, Mississippi, Louisiana, Arkansas and also Florida,

to a less extent. Much of the American literature has been inaccessible to me, yet from those references I have collected, it appears that not only is blackwater known in most of the Southern States, but that it is, at least in certain districts, quite a common disease. Thus HARE[5] found that fifty out of one hundred and fifty-four practitioners in those areas of the census which had a mortality of seventy (or over) per thousand other deaths see it frequently. Of these, Alabama provides four, Georgia nine, Mississipi fourteen, Texas twenty-seven. He mentions that COCHRANE, M.O.H., Alabama, collected six hundred and forty-two cases from different practitioners, with one hundred and fifty-eight deaths, or a mortality of twenty-five per cent. BAT SMITH[6] says it occurs in the interior of Louisiana, and in Alabama, dating from 1867, and in Texas. He also says it occurs in Central America and Brazil. FIELD[7] describes it in Virginia, where it is called Roanoke yellow fever, to distinguish it from yellow fever. KILPATRICK[8] says the real cause is undoubtedly malaria, and in Georgia is confined strictly to the white race. ROUP[9] says the disease, malarial hematuria, is quite common in the swamp districts of this State (Arkansas). BUSH[10] says the disease is getting to be a very common occurrence in Florida, Georgia, and the Mississipi bottoms.

In the report of the Tri-State Medical Association of Mississipi, Arkansas, Tennessee,[11] the following conclusions are arrived at :—

1. Coloured race not entirely exempt, among fifty-seven cases five in coloured persons.
2. Forty-one in males, sixteen in females.
3. Fifty-one cases occurred in persons who had been subject to malarial attacks for a greater or less time.
4. In thirty-six cases quinine had been given before the attack. In only one case had quinine not been given.

These instances, out of many, will suffice to shew that in the Southern States of America blackwater fever is a well-known, and apparently a common, disease. Numerous other instances will be found in the bibliography appended. We have then reference to its occurrence in the following eleven States, viz., Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Tennessee (?), Mississippi, Arkansas, Louisiana, Texas.

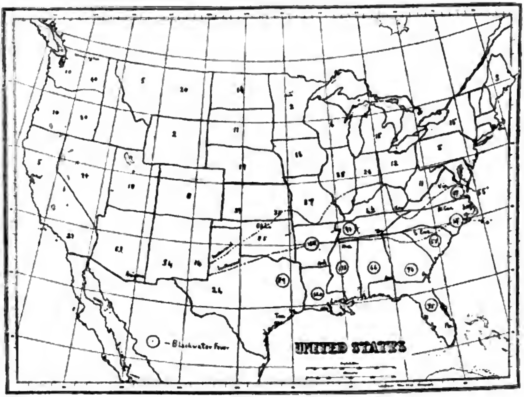
Let us next consider the distribution of malaria in the United States, and for this purpose I have used the data of the 1890 census. The figures represent the number of deaths from malaria per 1,000 other deaths. If we arrange the States in the order of their highest mortality, we get the following series (column 1): In the second, third, and fourth columns are given the values for other sections of each State, from which it will be seen how variable is the mortality even in the same State, *e.g.*, Georgia has in its southernmost part a mortality of seventy-four, in the northern part of only eighteen. Column 5 represents the occurrence of blackwater fever.

The following is the order of mortality from malaria per thousand deaths from other causes, and occurrence of Blackwater :—

	1	2	3	4	5
Arkansas	158	111	...	...	Blackwater
Mississippi ...	133	106	78	...	Blackwater
Louisiana	120	110	61	...	Blackwater
Tennessee ...	94	64	26	34	Blackwater ?
Texas	89	86	24	...	Blackwater
Oklahoma ...	85	...	...	...	...
Florida	75	...	...	...	Blackwater
Georgia	74	55	18	...	Blackwater
North Carolina ...	67	29	6	...	Blackwater
Alabama	66	62	41	...	Blackwater
South Carolina ...	58	46	43	...	Blackwater
Arizona	53	...	...	...	...
Virginia	47	34	11	...	Blackwater
Kentucky	46	27	25	15	...
Washington ...	40	10	...	...	...
Illinois	38	4	17	...	...
Missouri	37	36	25	20	...
Kansas	35	27	...	...	...
New Mexico ...	34	14	...	...	...
Indiana	24	15	...	...	...
Nevada	24	...	...	...	...
Etc., etc.... ...	...	...	...	...	...
Wisconsin ...	1	...	...	...	...

Thus with two exceptions we have an accurate correspondence between the distribution of blackwater fever and severe malaria. Whether Oklahoma and Arizona are real exceptions, or whether it is simply that blackwater, though existent there, has not been recorded, I have no means of determining. If now, we plot out these figures on the map, we see that blackwater fever is confined to a region below the 37th parallel,

probably 35th is more accurate. Further, the isothermal of 55° includes all these areas, while most of them lie within the isothermal of 60°. The mean annual rainfall is as great as fifty inches in Louisiana, Mississippi, Alabama, Tennessee, Georgia, and Florida, while in the rest it lies between forty and fifty.



Tennessee I have considered as positive, although I could find no actual statement regarding it, but I have inferred from the Tri-State (Alabama, Mississippi, Tennessee) investigation, of which an abstract only was available, that it occurs in Tennessee itself.

It is possible, too, that blackwater may occur in Kentucky, Washington, and a few others with high malarial mortalities. The data, however, shew that between blackwater fever and severe malaria there is, in fact, an extremely close correspondence.

Central America and West Indies.

References will be found in the bibliography to its occurrence in Central America,[58] Nicaragua,[59] Costa Rica,[60] Venezuela (Caracas,[61] and the Orinoco river[62]), and in Cuba.[63] The history of its occurrence in the West Indies[64] is an ancient one. Dutrouleau[65] says it occurs particularly in the most intense malarial localities of Fort de France, Martinique, and Point à Pitre, Guadeloupe. Only exceptionally at St. Pierre and Basse Terre. It was observed by M. L'Herminier as early as 1828-1838, and in 1853-1854. 'Continued longer, quinine treatment may be dangerous,

and at Point à Pitre they have not hesitated to attribute hemorrhagic complications to it, which were more frequent formerly than to-day.' It* occurs also in French Guiana,[66,67] British Guiana (uncommon), and Dutch Guiana (?)

South America.

BAT SMITH[6] records its existence in Brazil, but I can find no other reference. With regard to Central South America, the references in the literature at my disposal were quite inadequate to give any idea of either the prevalence of blackwater or of malaria.

Italy.

It is difficulty to gather from Italian literature what the exact distribution of blackwater fever in Italy is.

1. MARCHIAFARA and BIGNAMI[69] say, 'That in the Roman Campagna it is rarely seen. In the rest of Central Italy and in Northern Italy the only known case is the one published by MURRI. It is not rare in Sicily, and not very rare in Sardinia (Vincenzi).'

2. DE FRANCESCO MONTELONE[70] describes severe forms of fever in Calabria, seldom found elsewhere in Italy—pernicious hemorrhagic and tropical remittent (bilious).

* Corre[68] says that Duchassaing was the first to mention black urines arising from quinine in Guadeloupe.

3. With regard to the distribution of malaria, CELLI[81] says, 'Les parasites estivo-autunnales ont *un divers degré* de virulence et que en général, ils ont une virulence plus exaltée dans l'Italie inférieure, marennes de Toscane, et de Rome, Midi, Iles. Le nombre proportionnel des morts est beaucoup plus elevée dans l'Italie inférieure.'

4. *Vide* above, SCHAUDINN's observations with regard to the mildness of 'Tropica' (estivo-autumnal) in Northern Italy and Austria.

5. The accompanying map shews how markedly malaria varies in intensity throughout Italy, and how it reaches its maximum in the extreme South (Calabria), in Sicily and Sardinia, in both which places blackwater fever is 'not rare' (*vide* 1).

In these two instances, only, have I found it possible to compare the distribution of malaria and blackwater fever. In the United States the data shew a close correspondence; in Italy, also, we find that in the regions of really severe malaria, blackwater also occurs. Whether or no, the correspondence is as close as I believe the facts enable us to conclude, yet the varying intensity of malaria in the States and the striking variations in Italy also clearly shew that to speak broadly of malaria as a whole in a country is by no means justifiable.

In the rest of this paper I must necessarily be content with pointing out the distribution of blackwater, which is, I believe, wider than is generally supposed, adding only some comments which appear to be of interest, from the sources I have examined.

Greece.

PAMPOUKIS and CHOMATIANOS[84] say that quinine hemoglobinuria was first recorded by M. S. VARETTAS, Nov. 6, 1858 (*Soc. Med. Grécque de Paris*).

Blackwater fever is recorded by Greek physicians in Patras, Albania, Agrinion, the military hospital, Athens, Karvassara, Thessaly, Salonika, etc.

It is interesting to note that according to KARAMITSAS,[85] in Athens, malarial diseases have a very slight intensity, and are only seldom pernicious. With regard also to blackwater, KARAMITSAS[87] says, that no case of the disposition to blackwater is acquired in Athens.

Turkey.

KARAMITSAS records a case in a small village in Turkey. But here, as in so many other instances, it is impossible to form any idea of its real prevalence.

Russia (Merv).

GEKOW[95] investigated exactly, cases of blackwater fever that frequently occurred there. He takes the malaria and quinine view.

Palestine.

CROPPER[94] records blackwater fever in three districts. In *Khŭdeirah,* he says the natives suffer much less from malaria than the Jews, and hardly at all from

blackwater fever. At *Tantura*, the colony is now deserted on account of the severe malaria and blackwater fever. At *Isbeid*, blackwater fever also occurs commonly in some seasons. It occurs also at *Smyrna*.

Africa.

To treat of the history of blackwater fever in Africa, its distribution, is quite beyond the scope of this article, and would in fact require a treatise in itself. I can again only give short notes from the sources which have been available.

Soudan (Bahr el Gazal).

A death from blackwater fever was recorded from this region of Soudan, in the public press, August, 1902. I am informed that others have occurred since. CARMOUZE[96] records cases at Kayes, 15° N., 14° W., approximately.

Algeria.

KELSCH and KIENE[97] state that 'l'hémoglobinurie n'est pas exclusivenent liée aux fiévres bilieuses des pays chauds. Nous avons déjà signalé son apparation assez fréquente dans les fiévres d'Algérie, and also 'les pyrexies dans les quelles l'hémoglobinurie est le symptôme prédominant—sont dans la région Mediterranéene exceptionelles.'

VALLIN[98] records a case. It seems that blackwater fever is rare in Algeria. With regard to the distribution of malaria, or its varying intensity, I could find no data. With regard, however, to the species of parasite, BILLET[99] states that in Algeria, over a large area, the quartan parasite reaches a maximum value of only 2·5 per cent., but in Grande Kabylie it is 70 per cent. Such variations in the distribution of a particular species of parasite, in all probability, imply considerable variations, also in intensity of malaria, and it remains to be seen in what portions of Algeria really severe malaria occurs.

Senegal.

As it is commonly asserted by laymen on the West Coast of Africa that blackwater fever is a recent disease, it is interesting to note that in Senegal, according to BERENGER FERAUD[100], 'it has been observed as early as 1841, and from the resemblance of these histories to earlier ones, probably also in 1839, 1830, 1825, 1820, shortly after the arrival and settling of Europeans in Senegambia.'

Assini and Grand Bassam.

'M. LEGRAIN, 1850-1851, calls especial attention to the blackwater, stating that it was the only time he had seen it. Grand Bassam, 1855, three cases of "pissement du sang."'

Gold Coast and Gaboon.

M. Losedat, priest, had observed, since 1846, many times that malarial fever was accompanied by intense bilious phenomena, icterus, and black urine (p. 34[100]).

The frequency on the West Coast of Africa is given on p. 241[100] as—

Gaboon and Gold Coast	38-50	attacks	annually
Upper Senegal	20	,,	,,
Southern Rivers	15	,,	,,
Cayor	8	,,	,,
St. Louis and Gorée	1-3	,,	,,

That blackwater fever is common along the West Coast of Africa is a matter of common knowledge, but it is difficult to say where it is most frequent. One factor alone would make any estimation of the kind difficult, it is the very unequal distribution of a European population. Without Europeans there is practically no blackwater (its very rare, if genuine, occurrence among natives would, in no way, appreciably affect the statistics). Now, it is equally notorious that in the West Coast of Africa we have as regards its malaria one of, if not the most, deadly places in the world for Europeans. And this correspondence in the distribution of malaria and blackwater fever is not denied, of course, by those who deny their correspondence elsewhere. But I may be permitted to diverge so far from my intention not to base arguments as to their identity on the correspondence in distribution of blackwater fever and malaria, as to point out some striking facts with regard to the mortality from 'malaria' in tropical Africa. I examined the statistics of deaths in all German colonies in Africa for a number of years, and got these remarkable figures :—

Total deaths - European and native	Deaths from Malaria	Deaths from Blackwater fever
2948	8	62

Surely the conclusion that in these 'deadly fever-stricken regions' malaria has a comparatively small mortality is not the true one, but, on the contrary, the obvious one that blackwater fever is in fact malarial.

To resume the question of distribution, blackwater fever then is well-known along the West Coast of Africa, in Nigeria, in the Cameroons, in the Congo, probably Portuguese West Africa ; but whether as far south as Damaraland, I am unable to say. On the east coast it extends as far south as Beira and Delagoa Bay. British Central Africa, Uganda, German East Africa, British East Africa ; in all these it occurs. Cases will be found recorded in English periodicals. A consideration of Madagascar, Mauritius, the Comoro islands, may be interesting.

Madagascar.

DAULLÉ observed blackwater here in 1851-1854. DAVIDSON[104] describes cases at Nossi-Bé from 1862-1880, one hundred and eighty-five cases, forty-nine deaths. DUTROULEAU,[105] LEBEAU,[106] QUENNEC,[107] BARTHELEMY-BENOIT,[108] and YERSIN,[109] also describe blackwater in Madagascar.

Bourbon.

LAVERAN, quoted by MARCHIAFAVA and BIGNAMI,[114] says, 'the opinion that such a fever is produced by quinine is a popular belief among the créoles of Réunion, but one which is not shared by their physicians, who have always protested against the prejudice,' whereupon MARCHIAFAVA and BIGNAMI remark, 'but it seems that the prejudice was on the part of the physicians and that the laity were in the right'.

Mauritius.

HIRSCH and others mention its occurrence here. LABONTÉ discusses renal hemorrhage as a sequela of Mauritian fever. With regard to 'Mauritian fever' and the part played by quinine in determining blackwater, it is interesting to note that DE VALENCE[115] writes that, 'in 1823, JOSEPH CONISON said that sulphate of quinine *should be tried* in continuous fevers.'

India.

HIRSCH quotes DAY[117] regarding the occurrence of blackwater fever in India, but it is very doubtful if DAY's description refers to blackwater. Isolated cases have, from time to time, been recorded, especially from the Duars and Terai of Bengal, both notoriously malarial districts. CHRISTOPHERS and myself on visiting these districts found, to our surprise, as enquiry from Indian physicians had elicited, very little positive information on the subject, that in these districts (the tea gardens) blackwater fever was as common, and even commoner, than in tropical Africa.

In India it occurs then in the Duars and Terai (Bengal), in Assam, and in the Jeypore agency (Madras), though it is not at all improbable that it exists elsewhere also.* Regarding the distribution of malaria in India, extraordinary variations in intensity are found even within the limits of ten to twenty miles. In both the blackwater regions of India visited by us the malarial intensity was very high. (The malarial intensity or endemic index of a district is measured by the percentage of children, under ten, infected with parasites). In these districts it varied between 70 per cent. to 100 per cent. It was also a noteworthy fact that in both these districts the species of parasite was almost exclusively quartan, whilst elsewhere simple tertian was the prevailing species.

* Since writing the above Dr. Christy informs me that he has himself seen three cases in the Canara district (Bombay presidency). So that here, apparently, we have yet another focus in India.

China.

Cochin China. BERENGER FERAUD, *loc. cit.*, states that blackwater fever is frequent in Cochin China. HIRSCH, *loc. cit.*, VEILLARD,[119] DISSER,[120] record cases.

Tonkin. PAUCOT[122] saw seven cases in two years at Haut-Song-Cau. Five of these were among Europeans long in the colony.

FONTAINE[124] says, 'that blackwater fever is rare in the old *towns* of the Delta, but frequent in the high country, particularly at Dong Song.'

LE RAY[125] describes it at Cao-Bang.

Dutch East Indies.

V. LEENT[125] says, 'jusqu'a présent le fiévre jaune n'a jamais visité l'archipel des Indes Orientales. Nous croyons que ce sont des cas de fiévre pernicieuse ictero hemorrhagic qui ont conduit certains observateurs à admettre à tort l'apparition dans ces parages de cas de typhoide ictérode.'*

British Malaya.

HAMILTON WRIGHT[126] describing a case of 'pernicious fever' refers to it as rare. The case is undoubtedly blackwater fever, and is an accurate picture of that disease, though not recognized by the author as such.

New Guinea (German).

SCHELLONG,[127] DEMPWOLF[128] describe blackwater as existing here.

New Hebrides.

MOREL[129] describes a case. It may, however, be an imported one.

It must be evident from these scanty ones how inadequate are the references in literature to give a true idea of the exact prevalence of blackwater fever. The fact that in India, in the Jeypore agency (Madras), CHRISTOPHERS and myself found that practically all the missionaries established there had suffered from blackwater fever; that we had, in fact, a distinct blackwater area there which, at all events, to medical men outside the district was completely unknown, shows that caution must be exercised in assuming the absence of blackwater fever in a country unless it has been recorded. Nor do I think that the prevalence of blackwater fever in India is yet exhausted. Moreover, there is one fact which must be considered, viz., whether in any district we have a susceptible European population, and, in considering its relation to malaria, above all, differences in virulence of malaria must be taken into account, the species of parasite, and other factors, *e.g.*, conditions of life; thus it is a quite erroneous method in comparing the prevalence of blackwater among two European populations,

* Maurer[152A] states that there is no blackwater fever in Sumatra although the severest cases of malaria are not uncommon, but this is contradicted by the fact that it is common among the Dutch at Acheen. It also occurs at Java, especially at Tjilatjap (Scheube)[153].

to compare a town population with an 'out-station' population, for malaria among two such classes is a very different matter; it is in towns that malaria is least; it is the out-stations of the bush that it is most fatal. I have, I trust, avoided, as far as possible, mere argument in this paper, for argument and theories have been the bane of blackwater fever. I hope in a following paper to point out briefly what really are the facts which establish the malarial origin of blackwater fever.

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II. THE OCCURRENCE OF MALARIA PARASITES IN BLACKWATER FEVER

Analysis of Ninety-five Cases

PANSE has recently published a remarkable series of cases of blackwater, from which it is very evident that a positive or negative finding of parasites in blackwater depends very largely upon when the blood examination is made. This fact was already known to those who had made careful examinations in these cases, but I do not think that it is one that is at all generally recognized. I have, therefore, summarized all the cases, ninety-five in number, that I have been able to find bearing on this point.

I have confined myself to making clear this broad result, and have not considered many other interesting features of the cases, *e.g.*, (1) the data with regard to quinine, though quinine in itself is a powerful factor in causing the disappearance of parasites; (2) the actual intervals in hours between a positive and a negative finding; (3) the disappearance of parasites and their subsequent reappearance; (4) the number of parasites at various intervals till they disappear; (5) their occasional persistence throughout; (6) the presence of pigmented leucocytes; (7) the increase in the percentage of large mononuclear leucocytes (diagnostic of malaria). A consideration of such points as these would have much complicated the subject, and, indeed, the data are often quite inadequately given to make any such consideration possible, consequently I leave the tables to speak almost for themselves.

I have not considered cases where a clear history exists of the taking of quinine for several days previous to the blackwater, because simple malarial cases under such conditions may give an entirely negative result. Nor have I considered cases, a few in number, where a relapse occurred a day, or a few days at most, after the initial attack, because these cannot rightly be considered as fresh cases. Such cases, for instance, as those of KOCH's series, 13A, 13B; in these the quinine element on the contrary is clearly demonstrated. Finally, for the above reason, I have not included a series of cases of KLEINE, because, with one exception, the patient had been taking quinine for some days previously. The exceptional case was one where methylene blue had been given previously, and parasites were present; quinine was then given, blackwater ensued, and the parasites quickly disappeared.

The tables are constructed in the following way. The day before the blackwater, the day of the blackwater, and the day following, form three headings, and under each of these is recorded the result of the microscopical examination as regards malaria parasites, whether positive or negative (without further detail).

A. Plehn	Day before hemoglobinuria	Day of hemoglobinuria	Day after hemoglobinuria		Remarks
Case 1 ...	...	Positive	Negative	1	
„ 4 ...	Positive	...	Positive	2	
„ 6 ...	Positive	Negative	...	3	
„ 6 ...	Positive	...	...	4	
„ 7 ...	...	...	Negative	5	
„ 9 ...	Positive	Positive	Negative	6	
„ 17 ...	...	Positive	Negative	7	
„ 26 ...	Positive	...	Negative	8	
„ 27 ...	...	...	Negative	9	
„ 30 ...	...	...	Negative	10	
„ 31 ...	...	Negative	...	11	Blood examination before blackwater; not stated whether patient had been taking quinine.
„ 32 ...	...	...	Negative	12	
„ 35 ...	...	...	Positive	13	
Total ...	5	5	10		
Positive ...	5	3	2		
Percentage ...	100	60	20		

F. Plehn	Day before hemoglobinuria	Day of hemoglobinuria	Day after hemoglobinuria		REMARKS
Case 7 ...	...	Negative	...	14	
„ 8 ...	...	...	Positive	15	
„ 13 ...	...	...	Positive	16	
„ 15 ...	...	Positive	...	17	
„ 16 ...	...	Positive	...	18	
„ 17 ...	...	Positive	...	19	
„ 19 ...	...	Positive	...	20	
„ 21 ...	...	Positive	...	21	
„ 22 ...	...	Positive	Negative	22	
„ 23 ...	...	Positive	...	23	
„ 24 ...	...	Positive	Negative	24	
„ 25 ...	...	Positive	Negative	25	
„ 26 ...	...	Positive	...	26	
„ 28 ...	...	...	Negative	27	
„ 29 ...	...	Positive	...	28	
„ 30 ...	...	Positive	...	29	
„ 31 ...	...	Positive	..	30	
„ 35 ...	...	Positive	...	31	
„ 36 ...	...	Positive	...	32	
„ 37 ...	...	Negative	...	33	
„ 40 ...	...	Positive	...	34	
„ 41 ...	...	...	Negative	35	
„ 42 ...	...	Positive	Negative	36	
„ 43 ...	...	Positive	...	37	
„ 44 ...	...	Negative	Positive	38	
Total ...	...	21	10		
Positive ...	...	18	3		
Percentage .	...	85·7	33·3		

Koch	Day before hemoglobinuria	Day of hemoglobinuria	Day after hemoglobinuria		REMARKS
Case 4 ...	...	...	Negative	39	
„ 5 ...	...	Negative	Negative	40	
„ 6 ..	...	Negative	Negative	41	
„ 8 ...	...	Positive	...	42	
„ 9 ...	Positive	Positive	...	43	
„ 12 ...	Positive	Positive	Negative	44	
„ 12 ...	Positive	{ Positive { Negative	Negative	45	
„ 13 ...	Positive	Positive	...	46	
„ 13A...	[Negative]	[Negative]	[Negative]	...	
„ 13B...	[Negative]	[Negative]	[Negative]	...	
„ 17 ...	Positive	Positive	{ Positive { Negative	47	
Total ..	5	8	6		
Positive ...	5	6	1		
Percentage .	100	75	16·6		

Daniels	Day before hemoglobinuria	Day of hemoglobinuria	Day after hemoglobinuria		REMARKS
	Positive	Negative	...	48	
	Positive	{ Positive { Negative	Negative	49	
	Positive	Negative	Negative	50	
Total ...	3	3	2		
Positive ...	3	1	0		
Percentage .	100	33·3	0		

Stephens and Christophers	Day before onset	Day of hemoglobinuria	Day after onset		Remarks
Case 1 ...	...	...	Negative	51	
„ 2 ...	...	...	Negative	52	Numerous pigmented leucocytes day after onset.
„ 3 ...	Positive	Negative	Negative	53	Began as a typical case of malignant tertian, with numerous parasites.
„ 4 ...	...	...	Negative	54	Pigmented leucocytes day after hemoglobinuria. Crescents following day.
„ 4 ...	[Negative]	[Negative]	[Negative]	55	This was a relapse within twenty-hours, after quinine, of the previous case.
„ 5 ...	...	Negative	Negative	56	Pigmented leucocytes day following onset.
„ 8 ...	...	...	Negative	57	Pigmented leucocytes day after onset.
„ 9 ...	...	Negative	Negative	58	Much quinine on previous days; pigmented leucocytes day of hemoglobinuria.
„ 10 ...	...	Negative	...	59	Pigmented leucocytes day of hemoglobinuria.
„ 11 ...	...	Positive	Negative	60	Also pigmented leucocytes day of hemoglobinuria.
„ 12 ...	...	Negative	Negative	61	
„ 13 ...	...	...	Negative	62	Pigmented leucocytes day after onset.
„ 14 ...	...	...	Negative	63	
„ 15 ...	...	...	Negative	64	
„ 16 ...	...	Negative	Negative	65	Pigmented leucocytes day of onset.
„ 18 ...	...	Negative	Negative	66	Pigmented leucocytes two days after onset.
„ 19 ...	...	...	Negative	67	
„ 20 ...	...	Positive / Negative	Negative	68	At 3-45 p.m. many parasites (tertiana simplex) found, at 6 p.m. a single parasite only; shewing great importance of an early examination.
Total ...	1	9	16		
Positive ..	1	2	0		
Percentage .	[100]	22·2	0		

Panse	Day before hemoglobinuria	Day of hemoglobinuria	Day after hemoglobinuria		Remarks
Case 2 ...	...	...	Negative	69	
„ 3 ...	...	...	Positive	70	
„ 4 ...	...	...	Negative	71	
„ 6 ...	...	...	Negative	72	
„ 7 ...	...	...	Negative	73	
„ 9 ...	...	...	Negative	74	
„ 10 ...	...	...	Negative	75	
„ 12 ...	Positive	...	Positive	76	
„ 13 ..	...	Negative	...	77	
„ 14 ...	...	Negative	...	78	
„ 15 ...	...	...	Negative	79	
„ 17 ...	...	Positive	...	80	
„ 18 ...	...	...	Negative	81	
„ 19 ...	...	Negative	Negative	82	
„ 20 ...	...	Negative	...	83	
„ 21 ...	...	Positive	Negative	84	
„ 23 ..	...	Positive	...	85	
„ 26 ...	Positive	Positive	Negative	86	
„ 27 ...	...	Negative	Positive	87	Had been taking methylene blue for some days.
„ 28 ...	Positive	Positive	Positive	88	
„ 29 ...	Positive	Negative	...	89	
„ 30 ...	Negative	Positive	Negative	90	Parasites for three days previous to the first negative examination. No quinine, but taking methylene blue daily.
„ 31 ...	Positive	Negative	Negative	91	
„ 32 ...	Positive	Negative	Negative	92	
„ 33 ...	...	Positive	Positive Negative	93	
„ 34 ...	Positive	Positive	...	94	
„ 35 ...	Positive	Positive	Negative	95	
Total ...	9	17	20		
Positive ...	8	9	5		
Percentage .	88·8	52·9	25		

Author	Day before hemoglobinuria		Day of hemoglobinuria		Day after onset	
	No. of cases	No. positive	No. of cases	No. positive	No. of cases	No. positive
A. Plehn	5	5	5	3	10	2
F. Plehn	0	0	21	18	10	3
Koch	5	5	8	6	6	1
Stephens and Christophers	1	1	9	2	16	0
Daniels	3	3	3	1	2	0
Panse	9	8	17	9	20	5
Total	23	22	63	39	64	11
Percentage Positive ...	95·6 per cent.		61·9 per cent.		17·1 per cent.	

It is almost unnecessary to add any comment for the figures shew conclusively that when examined before the onset of the blackwater, parasites are present in 95·6 per cent. of cases, whereas on the day after, when it will be noticed the highest number of cases (sixty-four) was examined, the remarkable fall to 17·1 per cent. is the result. On the day of the blackwater itself, we have only a figure of 61·9 per cent. instead of 95·6 per cent. To assume, as has been done, that malaria in these cases is only an accidental concomitant seems to be to disregard common sense. My intention here is solely to draw attention to the great importance of an early examination, if malarial parasites are to be found.

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III. THE COMPARATIVE MORTALITY OF MALARIA AND BLACKWATER FEVER IN TROPICAL AFRICA

DR. CHRISTOPHERS and myself, while investigating the nature of blackwater fever, were much struck by the fact that in those intensely malarious parts of Africa visited by us, we did not encounter a single case of death from malaria, *per se*, but deaths occurred always from blackwater fever. The evidence that convinced us that blackwater fever is malarial, was, however, based entirely on microscopical evidence, and those who wish to know what the evidence really is may be referred to our reports quoted above. While maintaining then that the direct evidence of its malarial nature, even in the absence of parasites at the time when the cases are most frequently examined, is conclusive; it is not, I think, useless to consider evidence of a secondary nature. This evidence, too, is important, as it affords a mass of facts surely of much greater value than the many plausible suggestions made by those who have had no actual experience of the disease. To turn to the facts under consideration, it is not often that it is possible to give any numerical comparison of the frequency and fatality of malaria and blackwater fever respectively in a district. The following data I have compiled from the statistics furnished with regard to German colonial possessions in the 'Arbeiten aus dem kaiserlichen gesundheitsamte' for the various years. Frequently the data are insufficient to afford the necessary comparison, but the following examples are sufficiently instructive to merit compilation here.

In these statistics I have omitted the data (a few in number) with regard to native malaria, and the rare occurrence of blackwater among the native troops, because it is clearly absurd to consider European and native malaria as a whole and deduce any argument therefrom—the two conditions are so unlike. It must further be noticed that the number of cases under the heading blackwater cases may not be quite accurate, as in the reports it is only the deaths that are especially noted. However, this deficiency does not concern our present object. Further, under the heading malaria deaths, it is possible that some of these are due to blackwater, for it is not always clear whether the distinction has been drawn or not.

We have then the following striking figures:—

	Cases of Malaria	Deaths from Malaria	Cases of Blackwater Fever	Deaths from Blackwater Fever
G. E. Africa, April, 1897, to March, 1898	312	0	30	2
G. E. Africa, April, 1898, to March, 1899	345	1 (?)	33	3
G. E. Africa, April, 1899, to March, 1900	390	0	17	8 (or ? 11)
... ...	213	0	10	1
Cameroon, July, 1897, to June, 1898	138	1	12	2
Cameroon, July, 1899, to June, 1900	149	4	12	7
Cameroon, July, 1900, to June, 1901	186	0	28	8
Togo, 1899 to 1900	72	2	5	5
	1805	8 (? 7)	147	36 (? 39)

We see from these figures the striking fact that the number of deaths from blackwater fever is four and a half times as great as that from malaria, and that, excluding blackwater, malaria has a small mortality. But is this the true interpretation of the figures, which are drawn from countries where it has always been held that, if anywhere in the world, malaria is a deadly scourge? It is hardly necessary to point out the true explanation; that these malarial regions are so deadly because blackwater fever there, also prevalent, is likewise malarial.

Again, as in the previous papers, we will allow the figures and facts to speak for themselves. On one of the many plausible conjectures regarding blackwater, it may be well to make some remarks. The 'special parasite' theory of blackwater was suggested to those who saw in the fact that in blackwater fever and Texas fever of cattle, red or black urine occurs, a proof of their identity, and the conclusion naturally followed that in blackwater fever also a parasite, allied to piroplasma bovis, occurred, and in pursuit of this phantasy, writers have naturally also tried to implicate ticks in the causation of blackwater fever. Now, it may be definitely stated that there is absolutely not the slightest basis of fact in the whole of this fantastic hypothesis. The blood and organs of blackwater patients have now been examined by many competent observers, and nobody has ever observed any parasite of this nature—parasites which in Texas fever occur in enormous numbers, and are easily stained and detected. To dismiss this subject, we may yet consider whether a special variety of malaria parasite exists, quite another matter from the view we have been considering. It is proved beyond doubt that blackwater is malarial. Is there a special malarial parasite? This may be a difficult matter to determine, because, as we have seen, it is only before the onset of the hemoglobinuria that the parasites are constantly found. At present no evidence exists of any difference in morphology of the parasites found, but that a parasite is capable of displaying increased virulence under particular conditions we think there is much evidence to show. We cannot enter into this question further. We think that while it is possible the malarial parasites in blackwater may have special morphological characteristics, this is unlikely, and that the truer explanation is to be found in altered virulence depending on unknown 'climatic' conditions, *e.g.*, possibly the passage through a special species of anopheles, and upon changes in the constitution of Europeans not produced in temperate climes.

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SUMMARY OF RESEARCHES ON NATIVE MALARIA AND MALARIAL PROPHYLAXIS; ON BLACK-WATER FEVER: ITS NATURE AND PROPHYLAXIS

SUMMARY OF RESEARCHES ON NATIVE MALARIA AND MALARIAL PROPHYLAXIS; ON BLACK-WATER FEVER: ITS NATURE AND PROPHYLAXIS*

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I

Of our researches on malaria those relating to native malaria seem to us of such great practical importance that in the present report we have almost entirely confined ourselves to a discussion of these, and their application to the prevention of malaria among Europeans in the tropics.

It is unnecessary here to discuss the question of the mosquito transmission of malaria. We may, however, lay stress on some points which are as yet often overlooked.

1. It is practically certain that the mosquito cycle is the only one. We cannot, in the space at our disposal, give all the reasons for this statement: suffice it to say that up to the present no competent observer has brought forward a single fact inexplicable by mosquito transmission or suggesting any other channel of infection.

2. Regarding other hosts of the malarial parasite than man, no one has found any other animal than man infected with the organism of human malaria. The supposition that monkeys may be the means of infecting *Anopheles* in the jungle is made highly improbable by Koch's researches. We may point out, too, that such a supposition is in no way needed to explain malaria contracted in the jungle, the real mode of infection in such conditions being now quite well understood.

3. All recent research confirms the view that malaria is always derived from malaria pre-existing in others. It is as important to recognize that *malaria is only derived from man* as it is to appreciate that it is only transmitted by the mosquito (*Anopheles*). In other words, malaria is as much an infectious disease as scarlet fever,

* Slightly modified from *Papers Relating to the Investigation of Malaria and other Tropical Diseases*, Colonial Office, June, 1903. A summary of all our researches will shortly be published as a *Report to the Malarial Committee of the Royal Society*, Harrison & Sons, London.

the only difference being that it is not conveyed by contact, but only by the *Anopheles* mosquito.

Malaria then is an infectious disease—the infection or malaria parasite being conveyed from one person to another by the bite of *Anopheles*. An *Anopheles* mosquito, *per se*, is harmless ; it is only an *Anopheles* containing parasites, or, in other words, an infected *Anopheles*, that can transmit malaria, and the only way by which an *Anopheles* can become infected is by 'biting' some person who has the parasite in his or her blood.

It at first sight seems strange that the infective character of malaria has been and is still so overlooked by the general public. The actual mode of infection, however, has lately been made clear. Malaria is not in the tropics usually contracted from 'fever' cases, but almost always from 'latent' malaria in the native population.

Native Malaria

1. Koch, in the East Indies, almost everywhere found malaria in the children, though adults were free from infection. He commonly found malarial infection only on microscopical examination of the blood. In some of the villages examined by him every child had malaria, in others a smaller proportion. He came to the conclusion that the degree of infection he found in these children was a test of the intensity of malaria.

2. We, ourselves, finding that *Anopheles* caught in native villages always contained a considerable percentage of infected specimens, were led to the discovery that this was dependent upon a general infection of the native children of Africa who, although apparently in good health, had almost always the malarial parasite in their blood. We were thus able to show that the home of malaria is in native huts, hamlets, villages, and towns, and that European malaria is a mere resulting sign of the vast degree of indigenous latent malaria.

This has been amply confirmed subsequently by other observers, notably by Annett and Dutton in Nigeria, Ziemann in the Cameroons, and again by ourselves in India.

The Infection of Anopheles

Where human infection is so general we should also expect to find *Anopheles* infected, and, indeed, in any batch of *Anopheles* caught in native huts a greater or less proportion always contain sporozoits, *i.e.*, the malarial parasite in a condition ready for infecting man. The number of *Anopheles* infected is a variable one. As a rule in Africa it is from five per cent. to ten per cent., but reached in some villages fifty per cent. Aro, on the Lagos railway, was an instance where the sporozoit rate in *Anopheles* caught in native huts was fifty per cent. Obviously it would be as difficult to avoid malaria in such a place as smallpox in a smallpox hospital

We have said before that *Anopheles* are only capable of giving rise to malaria when they have previously fed upon blood containing malarial parasites, but a curious fact, and one of great importance, may be here noted. *Anopheles* are mainly to be found in association with native dwellings. One has not long to do with *Anopheles* without finding that they are hardly ever really abundant, except in native communities. Whenever in any place we wished to collect large numbers, our invariable practice was to proceed to the native quarter, and there we could collect *Anopheles* generally without difficulty. It was otherwise elsewhere, and in remote marshes occasional specimens (most frequently of a 'wild' species) only were caught in our tents. So far as appears at present, the majority of *Anopheles* in Africa haunt native villages. It is, perhaps, almost permissible to say that about five per cent. of all the *Anopheles* of tropical Africa are infected with malaria, and infected solely because they have derived the infection from latent native malaria.

In nearly every hut, then, of the millions scattered over the jungle lands of Africa, and of those forming the densely crowded towns of West Africa, we have children with parasites in their blood, and *Anopheles* to disseminate these.

The Consequent Infection of Europeans

The condition of extreme unhealthiness found, *par excellence*, in West Africa is not determined by the 'climate.' The reason is largely to be found in the conditions under which Europeans at present live in Africa. Even on general grounds it would be well to avoid native huts and hovels, with all their dirt and insanitary surroundings, conditions which may be likened to those in the worst slums of our large towns. When, however, we realize that these huts are veritable hotbeds of malaria, it is evident that the very first sanitary law for Europeans in Africa is to avoid their neighbourhood. It is, however, a striking, but most deplorable feature, that in Africa hardly ever do we find a European bungalow or dwelling place built with this end in view. European houses are often situated among the huts of the natives in towns, as in Freetown, Sierra Leone, or they have a cluster of hovels or huts close at hand. In one instance we saw a new settlement being built on the very fringe of a native village. It was not a question of necessity, as land free from villages or huts was available all around, nor was there any reason of policy, the Europeans being employed on the railway, and having no relation with the villagers. The choice of such a site sufficient in itself to ensure the settlement being a very deadly one, as indeed was later the case, could only be deplored. To sum up, then, we can say that with certain notable exceptions, to be mentioned later, the European on the West Coast of Africa is living in the midst of native huts, and is consequently daily exposed to the bites of infected *Anopheles*. The actual conditions are described in greater detail in the following section dealing with prophylaxis.

PROPHYLAXIS

At the outset we shall divide the prophylaxis of malaria under two heads:—

1. The prevention of malaria in native communities.
2. The prevention of malaria in Europeans.

The two problems are essentially different, and no confusion should ever exist in our minds as to what any given anti-malarial measures are intended to achieve, whether increased health of resident Europeans or diminution of native malaria.

1. The Prevenion of Malaria in Native Communities

Prophylactic measures applicable to the average native of tropical Africa are, for many years to come, beyond discussion. The vast bulk of African natives are completely beyond any sanitary control whatever. In some large towns a measure of control does exist, *e.g.*, Freetown, Lagos, Accra, Cape Coast Castle, and a few others. In these only has one the least hope of achieving any result.

Methods of malarial prevention applicable to large native communities seem confined to some form of *Anopheles* destruction, either by superficial drainage or by the continuous labours of a mosquito brigade. The administration of quinine, advocated by Koch, though so effective under the conditions at Stephansort, could not, we feel certain, be applied with any measure of success even in Freetown or Lagos. With regard to *Anopheles* destruction, if drainage can be carried out, it would, in our opinion, be successful in preventing native malaria.

2. The Prevention of Malaria in Europeans

In 1900, working on the Gold Coast, we advocated the protection of Europeans in Africa as being at present the proper and legitimate object of our limited resources in Africa. We gave reasons for believing that a system of segregation from the native, carried out as opportunity offered, would be far more effective than any other prophylactic measure within our power. We have already seen that the malarial fever to which the European is subject is due to the fact that he lives amidst the natives, or with the native at his door. We would emphasize again the fact that these conditions have impressed themselves upon us so vividly, because our experience of them has not been that of a passing observation, but one derived from actually living under them. We have enjoyed the hospitality of very many Europeans, and have slept in the bungalows and quarters of officials, railway engineers, missionaries, settlers, traders, in quarters in the centre of native camps, always with the inevitable native huts in the compound, and in all, these conditions held good. Realizing the danger of sleeping under such conditions, we succeeded in preserving our health only by most constant and unremitting care in the use of personal precautions. Such precautions we, however, found were generally so irksome that men preferred to run the risk of infection rather than bestow the necessary attention to them. Although we used mosquito nets we found it necessary to employ an extraordinary and troublesome degree of care in their use in such conditions as are usual in African up-country stations, and

we believe but few men would employ them with sufficient care to avoid infection in such places Similarly with regard to houses protected with wire gauze. Even where the oppressiveness of the climate would not preclude their use, we consider that it is only by a constant vigilance that but few men would exercise that such measures could be successful.

As a preliminary step to all other prophylactic measures, and as one likely more than any other to minimize European malaria we, therefore, advocate 'Segregation from the Native.'

Since we first put forward segregation as a principle to be followed whenever opportunity offered, it has been recognized by some authorities* as the first law of hygiene in the tropics ; on the other hand it has met with criticism. Much of the latter is evidently based on mistaken notions of what is meant by segregation, and what segregation entails. Segregation as an anti-malarial measure does not, for instance, mean the avoidance of intercourse with the native. Nor does it mean a lessening of the power of control of the native. It has been said that segregation means giving up a country. Such a notion could only arise from a complete misconception of what is meant by segregation in this connexion. The fact that in India segregation is almost universal seems to us to effectively meet such objections. In India we do not, except rarely, find European dwellings and native quarters crowded together, but almost always a well-designed European quarter, quite distinct from the native bazaar. Yet in India there can be no question of loss of touch with the natives—rather on the contrary, an increased respect on their part.

To talk also of the impossibility or impracticability of segregation in Africa is absurd, because a most excellently carried out scheme of segregation already exists at Accra (Victoriaborg), and to a less extent at Old Calabar, which places are noted on the West Coast for their comparative healthiness. Moreover, since we first advocated such measures they have been advocated also by ANNETT and DUTTON (Second Liverpool Expedition) as applicable, above any others, to Nigeria. These authors had actual experience, during many months, of the condition under which the European lives, and they advocate segregation as strongly as we do ourselves.

Further, LOGAN TAYLOR, himself engaged in destructive measures against mosquitoes, says,† 'When suitable ground can be had I think it better for the European to live away from the native town. In Accra the Government officials have good bungalows away from the native town, forming a proper European quarter (Victoriaborg), and this arrangement is found to work well, and Accra is the healthiest town on the Gold Coast.' ‡

* Manson. *Practitioner*, 1900. Annett and Dutton, *Second Expedition Liverpool School.*

† *Second Progress Report of the Campaign against Mosquitoes in Sierra Leone.* Liverpool University Press.

‡ That segregation is a practical measure there is increasing evidence to shew. Thus at Akassa (Nigeria) 'a hotbed of fever and disease,' segregation has been effected (together with drainage and the regular administration of quinine to Europeans), and Akassa is now one of the healthiest stations in West Africa. (*Brit. Med. Journ.*, April 18, 1903, p. 924.) Further segregation schemes have been carried out at Secondee and Cape Coast Castle.

PRACTICAL APPLICATION OF SEGREGATION TO VARIOUS CONDITIONS OF LIFE IN TROPICAL AFRICA

Since we feel very strongly that the segregation of Europeans is the first step in the prophylaxis of malaria in Africa and other tropical regions, we think it desirable to show how in practically every condition of life this principle can be applied.

So far prophylaxis (destructive measures) has been directed entirely against the malaria of large towns. If even, however, we could make the large towns of West Africa healthy we should still have an enormous fever and death-rate among Europeans on the Coast, quite half of whom live in out-stations, and the great majority of whom make very frequent tours. Moreover, it is in *out-stations* that Europeans are chiefly in need of protection. Whereas we found that residents in towns enjoyed, on the whole, fair health, it was in out-stations that we chiefly found men in the fever-stricken and miserable condition so characteristic of tropical Africa. We would point out then that something more than anti-malarial measures in large towns is needed if much improvement in the health of Europeans is to result.

It is here that 'segregation' holds out such prospects of success. Not only is the formation of a European quarter in large towns a fundamental law of health, but, as we shall show, in out-stations, railway camps, mission stations, in bungalows, in tea or coffee gardens, in expeditions, military or otherwise, in ordinary travelling, segregation is equally applicable.

Before malaria is made to decrease among Europeans in Africa it must be generally recognized that malaria is an infectious disease, and that it is present in practically every native hut. When this is the case men will refuse to allow in their compounds squalid grass and palm-leaf huts ; they will cease to build their bungalows among or on the outskirts of villages ; they will be extremely careful where they sleep when travelling, and it will be the duty of the medical officers of mining camps, railways, and military expeditions to absolutely forbid the forming of any camps near native huts, or to allow these to spring up in the more permanent camps.

1. Segregation in Large Towns

We have two noteworthy instances where in large towns segregation has been carried out most effectively, with the result that the two segregated communities, Accra (Victoriaborg) and Old Calabar, are notoriously the most healthy on the coast.

Moreover, in any large town where such complete segregation on a large scale is not immediately applicable, the principle should be borne in mind, and as opportunity offers, huts should be removed and European houses built in the open. Thus, at Lagos, a well-designed quarter could, we feel sure, be gradually formed, and would place Lagos in the same category as Accra. At Freetown, we believe arrangements are now being made to remove the official quarters to a segregated site on the hills,*

* Proposed site for European residences in the Sierra Leone hills. Stephens and Christophers. *Fifth Report to Malaria Committee, Royal Society.* Harrison & Sons, London.

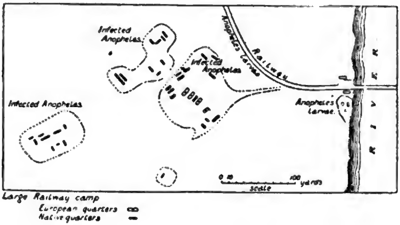
and at Cape Coast Castle also a site for officials has been chosen, well removed from the native town. The plan then to be followed in towns is the formation of a European quarter as distant as possible from native huts, and no better examples of this can be found than the arrangement in India of a European cantonment and native bazaar.

2. Segregation in Out-Stations

The terrible sickness and mortality resulting from the practice of the European living amidst native huts was nowhere more forcibly brought to our notice than in the camps of engineers and other employees engaged on railways under construction. Every settlement and every camp showed the same most deadly practice of allowing numerous native huts to be erected actually in the compound in which dwelt camp followers and their numerous families (children). To this fact alone is due the excessive sickness from malaria and, as a consequence, also blackwater fever on railways under construction. The working in swamps and the turning up of soil are in no way responsible, and a single instance will suffice to show to what railway engineers owe the malaria they so markedly suffer from, for never did we succeed in finding an exception to this deadly arrangement of native huts (with their constant fever supply) and European dwellings close at hand.

The Lokomeji Camp on the Lagos Railway (see plan):—

Plan of Lokomeji



About half-a-dozen Europeans lived in this enclosure. In it there were no less than thirteen native huts with, at a guess, one hundred inhabitants, men, women, and children.

This is more than enough to explain the constant sickness among Europeans here, but in addition there is a second crowded native compound, and a third within one hundred yards. When we consider that every one of these huts is a source of

malaria (for actually one-half of the *Anopheles* caught by us contained parasites in the stage ready for transmission to man) it becomes at once evident that a most unfortunate and disastrous mistake has been made by the Europeans living here. In such a case as this the engineer in charge of the section has the power of selecting a site, and has absolute power to forbid the building of any huts within any distance he chooses. When the European fully understands the certain danger to his health in living under such conditions, he will absolutely refuse to submit to such unnecessary exposure to danger. With ease, on a railway, a well segregated site for Europeans can always be provided. We shall see that a quarter to half-a-mile is ample, and some protection is ensured often by only a few hundred yards.

We have given a railway camp as an example of the evil effects of living amidst native huts because here, perhaps, most markedly did we see the result in the fever-stricken, anaemic Europeans, subject, more than any, to blackwater fever. The condition of life is, however, equally typical of *all out-stations*, the planter, missionary, trader, and even Government officials live universally under similar, though usually less deadly, conditions. In many such out-stations the condition could be remedied by very slight changes, the removal of a few hovels: often a single grass hut has been the source of perpetual fever among the Europeans living in a house.

We do not say that no native servant should sleep in a compound (though personally we found no inconvenience in allowing our servants to sleep away), for it is not in the presence of one or two adult servants that the danger lies, but in the numerous families (with children) crowded into the dark and dirty huts so universally seen at the European's door. Once the fact is recognized that it is from the crowded cooly lines and native quarters that the European derives his fever, we feel sure that the whole hygienic aspect of these fever camps will change.

Segregation on Military and other Expeditions and for Travellers and Sportsmen

The facts already put forward explain also why military expeditions in Africa are attended with such a large amount of sickness and so terrible a mortality. As an example of the mode of contracting malaria on military expeditions we may instance Prahsu, a well-known halting place on the way to Kumasi from the Coast. It is probable that every man passing up or down to the Coast during the Ashanti campaigns slept at least a night at this station. From plans of the condition there, European quarters and native huts in close proximity, it is at once evident that here at least was one of the sources of the sickness and mortality among European troops, and not in the 'climate.'

In native villages the native porters are able to procure food, and here generally a clearing is found ready for a camp. So that it is the almost invariable practice

of travellers of all kinds to camp in the village clearing, often to sleep in the native huts.

Here, again, we find the inevitable result. Although the traveller may not pay much attention to their bites, the *Anopheles* from the huts have injected malaria parasites (sporozoits) into his blood, and ten days later he is 'down with fever.'

Here, briefly, though it is a most important practical point, we must consider the question of flight of *Anopheles.* As the result of very numerous observations and experiments both in Africa and India, we may emphatically state that *Anopheles* do not often fly a quarter of a mile, and practically never half-a-mile. We are considering, it must be understood, the habitual, not the possible maximum, flight. *Anopheles*, in fact, tend to leave but for a short distance the thatched huts in which they spend the day, and although we give a quarter of a mile as a maximum flight, yet a segregation of one hundred yards from a native hut is infinitely better than none at all. Also, every natural obstacle tends to aid segregation, and should be taken advantage of, as the ridge of a hill, a belt of trees, bananas, bamboos, etc., and though a well-isolated dwelling, with no native families within a quarter or half-a-mile, should be aimed at, yet when native huts exist which it is impossible to remove, they should be as completely screened as possible by the planting of bananas, etc.

To sum up these various conditions we may say that a European who pitches his camp or builds his permanent quarters half-a-mile, to take an extreme limit, from any collection of native huts, however small, will avoid infection otherwise almost inevitable, and if in his compound he allows only those servants absolutely necessary, he is in a position to escape the dangers of life in tropical Africa.

PERSONAL PRECAUTIONS

We cannot emphasize finally too strongly the need at present for these. We, ourselves, by unremitting care, completely escaped contracting malaria during over three years' residence in Africa and India ; in places, too, where, more frequently than not, the deadly conditions we have described existed. Among these precautions we place the proper use of a mosquito net as by far and away the greatest means of individual protection.

1. *Mosquito net.*—The net should be square (not a bell net), should not have a single, even minute, hole, should hang inside the poles if these are used, should be tucked in *under* the mattress, and should *not* trail on the ground. A piece of closely woven material, fastened on all round at the level of the body is a necessary addition, in order to protect the limbs during sleep, from bites *through* the net. When not in use the ends of the net should be twisted up somewhat, and then thrown over the top. We always arranged our nets ourselves, never trusting to servants, and further, to be doubly certain, we always carefully searched the interior with a candle before going to sleep. To these minute precautions, solely, we attribute

our absolute freedom from malaria. Employed without care and attention, a mosquito net is little protection in such malarious places as most up-country African stations.

2. *Subsidiary measures.*—In many of the more malarious places we visited we considered that other subsidiary precautions, such as will suggest themselves to any intelligent person, were also necessary. To protect our legs and ankles, for instance, we considered it necessary to wear thick trousers, with puttees, or the very convenient so-called mosquito boots. The face and hands are not in waking hours very likely to be bitten by *Anopheles*, though they are very likely to be bitten by various species of *Culex*. It must be understood, however, that for precautions to be effective in badly malarious places considerable care and thoughtfulness is entailed, and few followed our example.

3. *Quinine.* During the whole of our three years' life in the tropics we found it quite unnecessary to use quinine. If, however, the bites of *Anopheles* cannot be guarded against, quinine should be taken as a prophylactic. We consider Professor KOCH's method of taking fifteen grains on two successive days in each week as the best. Repeated small doses are of doubtful efficacy.

II. BLACKWATER FEVER

Considering the fatality of this disease, and the fear it inspires in the European in Africa, we fully realized the importance of trying to solve the vexed question of its cause.

We believe that facts observed by us, based on direct microscopical evidence, have placed on an absolutely satisfactory footing its malarial nature. The importance of this has a great added value, because it follows that the prophylaxis is identical with that of malaria, and the European who can protect himself from attacks of malaria will have no fear of contracting blackwater fever.

It has been held by the majority of competent observers in recent years in the tropics, notably A. and F. PLEHN and ZIEMANN, that blackwater fever was malarial in nature. The most important objection to this view is that a microscopical examination of the blood in blackwater cases is generally negative, *i.e.*, shows no malarial parasites, or so few as to make it doubtful if they could be associated with the attack. This then was practically the state of our knowledge when we commenced our work on this fever.

In our investigations into the ordinary forms of malaria, however, in the tropics, we soon recognized that in severe malaria also, an examination of the blood might, in certain cases, reveal no parasites, or in other cases very few, quite insufficient apparently, to account for the severe symptoms. Such cases were those in which quinine had previously been taken, so that it was not an uncommon experience for a blood examination to show, before the taking of quinine, numerous parasites, whereas

later, while still high fever and severe symptoms continued, parasites might be entirely absent. Nor was it exclusively in cases where quinine had previously been taken that parasites were absent. In certain cases, though we believe these are comparatively rare, parasites may be absent, or if present, are so few in number that they bear no proportion to the severity of the attack. And, indeed, our experience has been confirmed by others working both in the tropics and in Europe, by CELLI, ZIEMANN, SCHAUDIN.* Thus the last named says, in case I, parasites were present during the attack and during the intervals of the fever, but in case II they were almost always absent on the day after the attack, and during the attack they were very scanty, although the fever was extraordinarily severe.

We were led then, in such cases as these, to seek for other proofs of the malarial origin of the fever. Two such methods were adopted by us: the first consisted in a thorough search in extensive blood films for pigmented leucocytes, which are evidence of a recent attack of malaria. The second was by a determination of the relative proportion of the different varieties of leucocytes in the blood. We followed out this line of observation at length, and found that in malaria a relative increase of the large mononuclear leucocytes took place, and were led to consider a value as high as twenty per cent. as evidence of an antecedent malarial attack. Similar observations had also led TÜRK, whose work was then unknown to us, to point out the diagnostic value of this increase in the diagnosis of malaria. We have, then, two auxiliary methods in the diagnosis of malaria:—

1. The detection of pigmented leucocytes.
2. The increase in the percentage of the large mononuclear leucocytes.

Applying these subsidiary tests to blackwater fever in which parasites are, as a rule, absent, we were able to show that nearly all of these cases, apparently non-malarial, are, in fact, malarial, presenting pigmented leucocytes and an increase in the large mononuclear leucocytes. In our first series of sixteen cases, although only in three were parasites found (about 19 per cent.), yet using these subsidiary tests no less than 93·7 per cent. were shown to be malarial.

We thus, as a result of our work, established, on a microscopical basis, the proof of what had been previously mainly conjecture.

Blackwater fever, then, is malarial in origin. It cannot, however, be considered as simply a severe form of malarial fever, for there is yet another side to the question.

In 1860, TOMASELLI first published a series of cases in which symptoms of blackwater fever followed upon the administration of quinine, not necessarily in large doses, but almost invariably in those who had suffered much from malaria.

A. PLEHN and F. PLEHN, in the Cameroons, have published most accurate histories of very many cases of blackwater, and with very rare exceptions they always followed upon the administration of quinine.

* *Arbeiten, a. d. k. Gesundheitsamte*, s. 234, Bd. xix.

Koch has so strongly advocated the quinine factor in blackwater that it appeared at first as if he denied its malarial origin, but this is not so, as is quite clear from his later writings. He holds that quinine is the immediate exciting cause, but that a predisposition, determined by many attacks of malaria, is necessary.

Many of those who hesitated to give their assent to these views now acknowledge that there is such a thing as quinine haemoglobinuria occurring in malarial cases. This acknowledgment amounts to a recognition of the quinine origin of blackwater fever, for the two conditions are absolutely indistinguishable. We have ourselves seen cases which were to us clearly of this nature, and our views are summed up by saying that blackwater fever is a disease malarial in origin, and dependent on blood changes occurring after many malarial attacks, and generally, if not always, in relation to an actual attack, but that, also, it is undoubtedly almost invariably induced by the taking of quinine in this state.

It has been argued that if quinine is the cause of blackwater fever it is a dangerous drug, and should not be used in malaria, but this argument is not a good one. It is, as we believe, in the malarial chronic that blackwater almost always occurs. We believe that in such a person quinine is dangerous. If, however, quinine is efficiently used as a preventive of malaria, no fear need be held of it. It is inadequate quinine treatment, because malaria is thereby not really combated, which is the danger.

While then we consider that the malarial origin of blackwater fever has been established by us on a basis of microscopical evidence, yet we may briefly consider some other aspects of the question, as it will enable us also to answer some of the objections of a purely general character, which have hitherto been raised against its malarial origin.

1. It has been urged that the distribution of blackwater fever and malaria is not the same. Even if this statement were true, it must not be forgotten that the distribution of mild malaria and severe malaria is by no means the same. Thus, the mortality from malaria in the few still remaining foci in Northern Europe is in no way comparable to that of the Roman Campagna, nor, again, is the severity of malaria in Northern Italy comparable with that in the South. We cannot, indeed, speak of the distribution of malaria as a whole. If, however, we confine our remarks to regions of intense malaria we believe that the distribution of blackwater fever will be found to tally exceedingly closely with that of malaria. The distribution of blackwater fever is, we believe, considerably wider than is generally supposed. Thus in the Bengal Duars, in India, blackwater was found by us to be as common as in Africa, a fact to which the literature of the distribution of blackwater fever gave us no clue.

Further, in Madras we discovered the existence of blackwater fever, the existence of which was quite unknown, even to medical men in India. We believe, in fact, that there is rather a very close and even exact parallel between the distribution of blackwater fever and severe malaria.

2. An accurate history of blackwater fever cases will always reveal that the patient has suffered more or less constantly from previous attacks of fever, and that for a day or two previous to his attack he has had a more or less markedly high temperature. That this temperature is of malarial origin is shown by microscopical evidence, for,

3. If the blood of a patient about to suffer from blackwater fever is examined by chance before the onset of the disease, and before the taking of quinine, it is almost invariably the case that malarial parasites are easily found. An examination of the same case after the onset of the blackwater is, however, most frequently negative as regards parasites. Thus PANSE,* in a recent paper, found parasites without exception in all those cases which he was able to examine immediately before the onset of the haemoglobinuria; and arrives at exactly the same conclusion as ourselves as to the direct dependence of blackwater on malarial infection.

4. That blackwater fever affects residents mainly in their second and third year suggests that it occurs in conditions of chronic malarial infection, and is strongly against a view which has been suggested that blackwater fever is due to a special parasite. Thus BERENGER-FERAUD† gives the following data: First year, 5.4 per cent.; second year, 22·5 per cent.; third year, 42·5 per cent.; fourth year, 20 per cent.; fifth year, 4·8 per cent.

5. Again, the fact that, in West Africa and other regions where blackwater fever occurs, Europeans die not so much of malaria but of blackwater fever seems to admit only of one conclusion. To give more exact figures, it appears that in the German Colonial possessions, out of 3,000 cases of malaria, there were eight deaths only from ordinary malaria, but sixty-two from blackwater fever. However we consider these general points they all clearly point to the malarial origin of blackwater fever, though, as we have said, the real evidence depends upon the microscopical evidence of malaria in blackwater.

Blackwater fever, then, is malarial in its nature, and its prophylaxis is consequently identical with that of malaria.

* *Zeitschrift fur Hygiene*, x. i, 1903

† *De la fièvre bilieuse melanurique des pays Chauds.* Paris, 1874

REPORT OF THE
MALARIA EXPEDITION TO THE GAMBIA

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR X

REPORT

OF THE

MALARIA EXPEDITION TO THE GAMBIA

1902

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY

J. EVERETT DUTTON, M.B., B.CH., VICT.

AND AN

APPENDIX BY F. V. THEOBALD, M.A.

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I. PRELIMINARY

DURING the past two years a great deal of energy has been displayed in many

ERRATA

In acknowledgment, line 11, for 'Lieut. G. C. YOUNG,' read 'Lieut. G. C. TRACY.'

P. 4, l. 23. For 'regular,' read 'irregular;' for 'rarely,' read 'readily;' for 'up,' read 'off.'

P. 9, l. 33. For 'Lieut. YOUNG,' read 'Lieut. TRACY.'

P. 10, l. 19. For 'Mr. BARNOIS,' read 'Mr. BUDGETT.'

P. 10, l. 24. For 'cus cus,' read 'koos-koos.'

P. 10, l. 34. For 'Lieut. YOUNG,' read 'Lieut. TRACY.'

P. 21, last line. For 'ground,' read 'drain.'

P. 26, l. 21. For 'mosquito' read 'mosquitoes.'

P. 33, l. 22. For 'mosquito-room,' read 'anti-mosquito-room.'

P. 34, l. 5. For 'country,' read 'community.'

P. 34, l. 37. Before 'water,' read 'tidal.'

P. 36, l. 36. Professor HERDMAN has recently kindly identified for me the specimens of fish taken from the main drains of Bathurst; two specimens so far occur. They are:—

Hemichromis bimaculatus (GILL).

Chromis macrocephalus (BLEEKER).

P. 39, l. 30. For 'great,' read 'greater.'

not necessarily require such specialized breeding places, for if from any cause they were absent these mosquitoes would breed in any collection of water, just as the genus *Culex* does.

REPORT OF THE LIVERPOOL MALARIA EXPEDITION TO THE GAMBIA

I. PRELIMINARY

DURING the past two years a great deal of energy has been displayed in many parts of the world to put to some practical use the great discovery of the definitive host of the malaria parasite. The knowledge that the mosquito is responsible for the transmission of malaria has immensely increased the possibility of combating this disease by prophylactic measures. The measures which have been recommended by various investigators naturally group themselves under two heads—

1. Destruction of the parasite in its intermediary host, man.
2. The prevention of the transference of the parasite from one host to the other, viz., from man to mosquito, or mosquito to man.

Under the latter group many methods have been advocated, and much literature has accumulated during the last three or four years. The modes of attack have centred round the vulnerable stage in the life cycle of parasites in general, viz., the transference from one host to another; thus in the case of the malaria parasite the points aimed at in this stage are—

1. Destruction of the definitive host, the mosquito.
2. The prevention of infection of the mosquito.
3. The prevention of inoculation of man by the mosquito.

It was hoped in the early stages of the work of investigation on the mosquito cycle of the malaria parasite that it would be a matter of comparatively little difficulty to destroy the parasite in this stage of its history by means of the destruction of the mosquito carrying them, especially as only one genus of the *Culicidae* (*Anopheles*) was implicated. Major Ross, in India, and other investigators had pointed out the broad fact that the genus *Anopheles* required, as a rule, breeding-places of a rather special character for their propagation, very shallow surface pools of sufficient depth to last over a week. By the prevention or treatment of these pools it was thought that the mosquitoes could be done away with. Maps were made marking out the distribution of such breeding places throughout certain districts with this object in view. Later on it was shown that certain members at least, if not the whole genus *Anopheles*, did not necessarily require such specialized breeding places, for if from any cause they were absent these mosquitoes would breed in any collection of water, just as the genus *Culex* does.

Up to 1899 some one hundred and twenty-two definite species of the *Culicidae* were described. Since that time many collections of mosquitoes have been examined from all parts of the world. Mr. F. V. THEOBALD, at the close of last year, as the result of an exhaustive examination of past works and new material, has remodelled the classification of the *Culicidae*, which he has grouped into some twenty-two genera, and has described some one hundred and thirty-six distinct new species in his *Monograph on the Culicidae of the World*. To this number must be added some one hundred new species which he has not yet described.

As yet only a few of the great number of mosquitoes have been investigated with regard to the important point of whether or no they can act as hosts for the malaria parasite. In fact only a few species of *Culex* have been shown to be incapable of transmitting the disease.

When it was considered that not only malaria but other diseases were conveyed by mosquitoes, for example, filaria can develop in two genera, *Culex* and *Panoplites*, and that yellow fever is certainly carried by one genus, *Stegomyia*, there arose a tendency to look upon all mosquitoes as harmful, and that their judicial destruction, as far as possible, was an object to be aimed at. How far this end can be attained is at present *sub judice*, but we have evidence that certain districts and towns lend themselves readily to this object.

Experiments under this head are at present being carried on in West Africa and in Havana. On a smaller scale similar experiments have been carried out in Hong Kong and Statin Island, New York. Major Ross chose Freetown, Sierra Leone, for the scene for an experiment, to see how far mosquitoes can be diminished in a certain area, one of the most difficult places on the West Coast to tackle. He and Dr. LOGAN TAYLOR, who was to direct the work there, arrived on July 2, 1901, and at once commenced the campaign against all varieties of mosquitoes. As yet the experiment is not completed, but a great diminution in the numbers of these insects has already been brought about.

In Havana the work of exterminating mosquitoes, by the destruction of their breeding places, was commenced soon after the discovery that yellow fever could be transmitted by the bites of the common mosquito, *Stegomyia fasciata*. Major and Surgeon W. C. GORGAS, in his January report to the Military Governor, states that out of seventeen thousand houses examined during the month by the 'Stegomyia Brigade,' in four hundred and eleven only were mosquito larvae found ; the preceding January, larvae would have been found in all of the houses. Not only have the mosquitoes diminished, but Havana has been for the last four months, October, November, December, January, entirely free from yellow fever. This result is certainly very encouraging when we compare the prevalence of yellow fever there during the same period of the previous year, when the average number of deaths from this disease was 146·49.

II. TOPOGRAPHY AND STATISTICS

St. Mary's Island and Bathurst

St. Mary's Island is a long, low-lying island at the mouth of the River Gambia, extending roughly from north-east to south-west along the south bank of the river. It is about four miles long and half-a-mile across at its widest part. The island is only separated from the mainland by a small creek, which, at the north-west corner, is bridged across. One fairly good road runs the length of the island, from the town of Bathurst at the one end to the Creek Bridge. This road runs along the beach, separated from it for the most part by mounds of sand and scrub. On the south side of the road an extensive mangrove swamp occurs, and in places encroaches on to the road.

The town of Bathurst is situated at the east corner of St. Mary's Island. It occupies an ⊥-shaped area of land, the long arm of which is almost separated from the island by an encroachment of Oyster Creek at the back of the island and the mangrove swamp. There is only a distance of some two hundred yards between the swamp and the beach, so that this area is for the most part cut off from the island, and is surrounded by a broad expanse of water on all sides, to the north and west by the River Gambia, which is here about four miles across, to the south and west by the creek separating St. Mary's Island from the mainland. In this area the land scarcely reaches the height of four feet above sea-level, the greater part of it being situated below the sea-level. Altogether it is scarcely a square mile in extent.

The formation is of light sand and loam on the surface, followed by denser loam which rests upon the water-bearing sand and silt, about eight feet under the surface. This area is well though not too thickly wooded ; some very fine trees occur in the town.

The town of Bathurst is very well laid out. The chief streets are broad and run parallel and at right angles to one another ; in fact there are very few narrow streets, even in the strictly native quarters. The chief houses and factories in the principal street, Wellington, face the mouth of the river. These houses are built of stone for the most part, the volcanic iron stone which occurs in great quantity across the river. These houses are very cool and airy, and the rooms are large ; they were probably built by the French. At the back of each house there is generally an enclosed piece of ground used as a garden.

Unfortunately, in Bathurst, the houses of the Europeans are not segregated from the natives, many of the traders' and officials' houses, particularly, being surrounded by native compounds. Government House, the Colonial Secretary's house,

the Hospital, and the Telegraph Station are slightly better in this respect, but here only a road (Clifton Road) and an open space about one hundred yards across separate them from a bad portion of the native town (Portuguese Town), and on this portion of land a few huts are present.

In the centre of Bathurst there is a fine open space, McCarthy's Square. On one side of this square is a very picturesque building, the Barracks, occupied during the time I was at Bathurst by a West Indian Regiment. The creek separating St. Mary's Island and the mainland commences at the back of the town of Bathurst ; and here it is about half-a-mile to a mile across. The ground in this part of Bathurst is very low-lying, and parts of it are below sea-level. To prevent the encroachment of tidal water a wall two to three feet high has been built. This wall extends for a considerable distance, the remainder being a mud embankment. Within this J-shaped area of St. Mary's Island described above two large swamps occur, namely, Half Die and Box Bar. The former swamp is situated in the smaller arm and is not tidal, owing to the low wall mentioned above. Native compounds occur all along these marshes, which in the wet season are often covered with water. Down the centre of this swamp a wide channel runs and opens into the creek by means of sluice gates.

The other swamp, Box Bar, is situated at the back of the town, in the larger arm of land ; along its centre an open drain runs, which falls in a similar manner by means of sluice gates into the creek. On either side of this swamp the native town extends for some distance, as Portuguese Town on the one hand and New Town on the other. This swamp is larger than the preceding one and is partially covered by rank grass and low scrub. Its surface is much more regular, so that the collections of water are rarely shut up from the central channel ; there appears to be a distinct fall, though slight, from the sides to the centre. Both of these swamps in the dry season only contain tidal water, which oozes through the sluice gates and sand into the central channel. In the wet season they practically act as reservoirs for rain-water. There is, besides, another smaller swamp.

Population of Bathurst.—The European population of Bathurst varies slightly during the year, some of the officials and traders returning home during the wet season. There are some seventy to eighty persons. The native population is estimated at about fifteen thousand ; they include the Joloffs, Mandingoes (Mohammedans), and a small number of Jolahs (Pagans), and a good few Sierra Leone traders. Besides this there is a small fluctuating population of Assyrian traders, estimated at about one hundred. These people trade in small articles among the natives, and live in an extremely filthy condition. A few cases of yellow fever which occurred in Bathurst last year were supposed to have arisen by means of these people.

Drainage of Bathurst.—Provision is made for the carriage of surface water by means of open gutters, which run down the centre of the main streets. These open channels commence at the higher portion of the town as shallow gutters, about

eighteen inches deep. During their course along the streets they are made deeper in order to obtain a good fall until they reach the beach, where some are thirteen feet deep and three feet wide. Similar drains from the side streets enter at right angles into these drains; the water from them is discharged into the river by means of sluice gates, which are opened at low tide. Altogether there are about half-a-dozen such gates. The construction of the drains varies to a considerable extent. The main drains opening into the river are square, flat-bottomed, built of stone, lined with cement, and are for the most part impervious. Further in the town the channels are built with stone, but some of them are only partially cemented, either the iron-stone blocks on the sides or the bed of the drain being uncemented. These channels allow the ground-water to percolate into them at certain seasons of the year. Still further in the centre of the town the drains become very shallow and are made of bricks, trough-shaped and uncemented.

Many of the streets have no 'made' drains as described above, instead, a centrally situated trench is dug in the ground, which either discharges into the main drain or discharges at the back of the town, directly into the swamp. These trenches vary considerably in depth and width, some of them being four feet from the surface of the ground. In some of the drains the earth dug out to form the trench is built up on either side of it so that the surface of the street falls away from the trench. Similar trenches occur round compounds, squares, and in the grounds of the Europeans. They are for the most part choked with grass, or natives walking across from one side of the street to the other carry with them sand or débris, converting the drain into a series of holes in the centre of the street; some of them are so choked in this way along their course and at their outlets that it is impossible for the contained water to escape. At the corners of the principal streets these drains are bridged over by low brick arches. In this situation the drain frequently has a stone placed in its course or a cemented catch-pit has been constructed; here water lodges for a considerable period after the rain has ceased. Later on it will be shown how many of these drains afford considerable breeding-grounds for mosquitoes.

Water Supply.—Rain-water is used for drinking purposes by the Europeans, and also by some of the native traders. It is gathered from the roof and stored in large tanks. Some of these tanks are fitted with good covers, others are not covered properly, so that insects, dirt, etc., contaminating the water, obtain an entrance beneath the cover of the tank. The best tank seen was one in which the only mode of access to the tank was by means of a man-hole at the top, with a heavy, fluted, iron lid, difficult of removal. Some of the native traders also collect rain-water in large iron boilers or tubs. These are for the most part inadequately covered, or not at all. For domestic purposes, water is obtained by the Europeans from wells sunk in their gardens. The native population of Bathurst obtain water solely from wells.

The wells of Bathurst are of two types, namely, deep wells and shallow or tub wells.

The deep wells occur as public wells, built by the Government. In the streets in various parts of the town there are about a dozen altogether. Similar wells are present in some of the European and native traders' compounds. They are on the whole well built of stone and steined with cement for a good distance down. A wall two feet high generally surrounds the well, but unfortunately very few of them are covered in. Of fifty-five private wells examined only thirteen were properly covered ; of the public wells, one only. Thus it is an easy matter for these wells to become foul ; this is generally due to sticks, half-peeled oranges, pawpaw rinds, and other rubbish thrown in by children. The depth of these wells varies from fifteen to twenty feet. The second class of well, the small tub well, occurs chiefly in the native compounds and in the gardens of the Europeans. They are very shallow, a hole being dug about three feet in the ground by a native and a barrel inserted into it, in which water collects. The rim of the tub may be flush with the ground, but it is generally about four to six inches above. Close to these small wells in the native compounds, in many instances scarcely four yards away, occur the cess-pit or midden and the screened-off personal wash-place. It is not difficult to understand how easily polluted these wells become. Many of the natives appreciate this, and on enquiry will tell you that they go to the public wells to get water for drinking purposes.

Climate of Bathurst.—With regard to climate, Bathurst represents a contrast to other parts of the Coast. Its situation on an island surrounded almost on all sides by a broad expanse of water tends to modify the extreme heat and dampness met with in other parts of the Coast. The dry season commences at the end of October and ends at the beginning of May. During these months the harmattan blows from the interior, and great variations between the maximum and minimum temperature may be recorded, a temperature of 100° during the day, while at night it is not unusual to have a temperature of 60°. These months are very trying to the native population, and many cases of bronchitis and pneumonia occur amongst them. During this season practically no rain occurs. The wet season sets in in June and ends in September. In the months before and after this period, that is April and October, some heavy showers may occur. In these months the temperature is much more even. The highest maximum recorded in the shade never reaches 100°, at least has not done so for the past five years ; it is generally about one or two degrees above 90°. The lowest minimum temperature recorded for these four months keeps about 70°. Chart (I) gives a record of the temperature of the maximum and minimum for 1900. For the last five years charts constructed in a similar way show practically the same curves. The rain-fall of Bathurst does not vary much above fifty inches, and this occurs in the four months, June, July, August, September. In October the wet season ends. After October rapid drying takes place. The wet season, owing to the great humidity and absence of any appreciable variation in atmospheric temperature, is exceedingly trying to Europeans.

Food.—Europeans at Bathurst are fortunate with regard to fresh food ; fresh meat can be obtained every day. There is a special slaughter-house for cattle, situated behind the market, which is under the supervision of the Sanitary Board. Fish is brought into the town twice daily, and is of excellent quality. Very good bread can be obtained from the various traders, particularly the French Companies ; the smaller native traders also sell bread in the market. Throughout the dry season English vegetables of all kinds are easily obtainable. Many of the European officials and European traders make a point of growing these vegetables, and I observe that the natives are, in a small way, imitating the Europeans in this respect. Many of them grow onions and tomatoes to sell in the market. Through the energy of the French Company ice can be obtained all the year round.

Disposal of Refuse.—Amongst the Europeans, sand closets are almost universally used, being emptied every day by a staff of night-soil men. With regard to the natives, public latrines are provided by the Government. At times it happens that excretal matters are washed by the tide on to the foreshore. In the compounds of the more wealthy class of natives middens are used ; some of these are of very large size, and are not made impervious to water. In the smaller compounds a tub placed in the ground is used as a privy. Only occasionally earth or lime is mixed with the excretal matter. When full, which takes from one to two years, the tub is discarded and another inserted in a fresh place in the compound. Both the tubs and privies were found to be infested with multitudes of fly maggots. The Jollofs, who constitute about one-third of the population of Bathurst, have no middens or tubs in their compounds ; instead, an earthenware jar is used to retain excreta, the jar being emptied every day, either in the morning or at night, into the river. These people are particularly clean and tidy and take a pride in keeping their compounds in good order. Dry refuse is removed from the various compounds by the Sanitary Board's carts, which go round the town every day ; this refuse is dumped on the borders of Box Bar swamp.

Prevalence of Malaria Fever in Bathurst.—With such a comparatively small and fluctuating white population in Bathurst it is difficult to estimate exactly the prevalence of malarial fever amongst the Europeans. From the medical officers' reports, which the Colonial Surgeon, Dr. R. M. Forde, very kindly allowed me to consult, I was able to obtain the following data for the last three years :—In 1898, out of a total European population of sixty-three, twenty-three persons were admitted into the hospital for various diseases, but principally for malarial fever. There were three deaths for the year, two from haemoglobinuric fever and one from malarial cachexia; during the year there were four cases of blackwater fever amongst the non-official European residents. In 1899, out of a population of eighty, twenty were admitted during the year into the hospital, chiefly for malarial fever. Of the non-official white population sixty-six were treated during the year ; of these thirty-seven were cases of malarial fever of the remittent type and three were cases of haemoglobinuric fever ;

there was one death from cardiac failure after enteritis. In 1900 there were eleven Europeans in the hospital ; two deaths occurred, one from dysentery, the other was a sailor landed in Bathurst with an abscess in the brain. Of the non-official Europeans sixty-nine were under medical treatment ; of these thirty-five were malarial fevers of the remittent type, with one death ; seven were believed to be yellow fever, with six deaths. It is stated in the reports that the severe cases of fever occur during the latter part of the year, from July onwards, that is, after the wet season has fully set in. In connexion with these figures the Colonial Surgeon points out that a fairly large percentage of Europeans return to England during the wet months. During the past year, 1901, there has been stationed in Bathurst a company of the West Indian Regiment. These men, though drafted from Sierra Leone, had for the most part originally come from Barbadoes and other West Indian Islands where endemic malaria does not exist, thus they were in the same category as the white man entering an endemic malarial district, and were therefore specially suitable to study the liability to infection with malarial fever. Further, no special precautions were taken amongst them to ward off attacks from mosquitoes, nor was quinine administered as a prophylactic. The men arrived in April, and were quartered in the barracks, McCarthy Square. Some of them went away for a short time in the early part of the year on a punitive expedition against some tribes up the river ; altogether there were one hundred and eight men. The following chart shows the percentage of cases amongst

(Chart I)

this force admitted into hospital with malarial fever during each month from April, 1901, to January, 1902, indicated in the chart by the thick line. The fever was for the most part remittent in character, and the diagnosis was to a large extent confirmed by microscopical examination of the blood. The curve does not represent the total number of malarial fever cases occurring amongst the soldiers, as many soldiers suffering from only slight attacks of fever were not ill enough to be admitted into hospital. With this chart is also given the rainfall in inches, and the maximum and minimum temperatures in the shade for the year. It will be seen that the greatest percentage of malarial cases occurred in the months of September, October, and November, and that there was a marked decline from December to January ; in the latter month no cases occurred. During the former months innumerable mosquitoes are present in Bathurst ; the rainy season being fully established during July and August has so raised the level of the ground water that many suitable breeding-places occur along the borders of the swamp and in the drains, etc. Besides the old observations illustrated in this chart of the relation between rainfall and malarial fever, it is also interesting to note that the greatest percentage of malarial fever cases amongst the soldiers also occurred in those months when the variations in atmospheric temperature are least marked, namely, July, August, September, October. The average variations between the maximum and minimum temperatures in the shade in these months

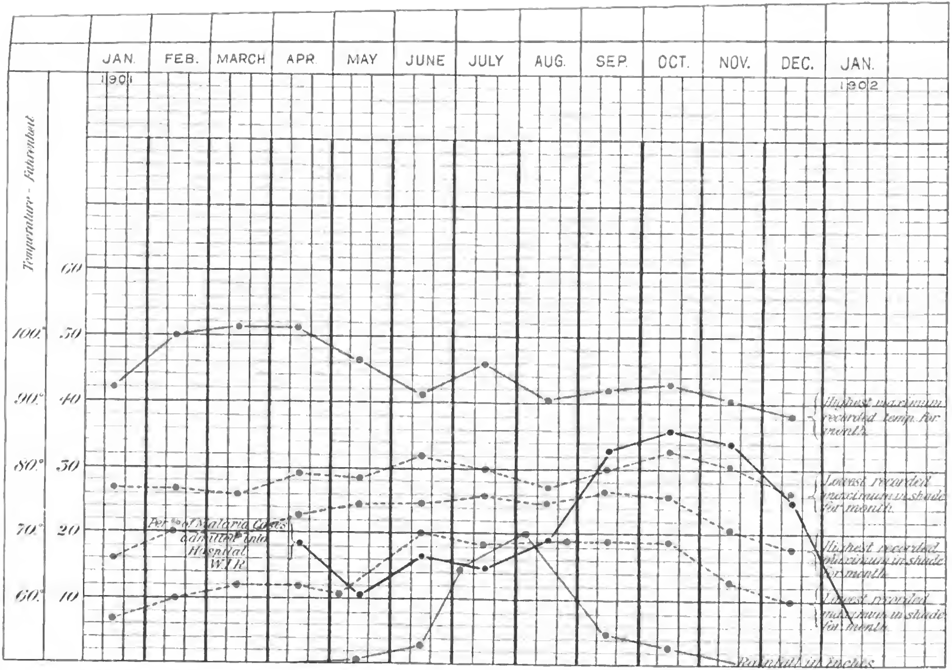


Chart I. Fever cases amongst the West Indian Soldiers stationed at Bathurst with rainfall and temperature curves for year:

being 25°, while in January, February, March, April, and May the variation in temperature is not less than 40°; it would appear, then, as pointed out by Dr. R. M. Forde in his Medical Report, that the liability to infection occurs soon after the rains are established, lasting up to the end of November.

Cape St. Mary

About seven miles from Bathurst, on the mainland at the mouth of the River Gambia, is a promontory known as Cape St. Mary, or simply the Cape. The road from Bathurst leads directly to it, and is perfectly level until a distance of about three hundred yards from the Cape is reached; the ground then rises rather suddenly to the summit of the promontory about one hundred feet. Cape St. Mary is the termination of the low cliffs of volcanic and sandstone rock which skirt the sea border to the south of the River Gambia. I had an opportunity of staying there two days in the Government House, which is built on the edge of a cliff facing the sea, and is now used principally as a convalescent station. Besides Government House, a few traders and others have built bungalows here and there along the cliffs. On either side of the road leading to Government House, and extending from right to left, is a large Mandingo town, and at the foot of the hill itself is an extensive swamp in which the natives grow their rice. There is no doubt that the climate at the Cape is very bracing and very beneficial to convalescents after fever. It is extremely unfortunate that Government House is situated so near the native village, in which endemic malaria and other diseases are very prevalent, and also that the rice swamp is so close at hand, breeding millions of mosquitoes, which not only cause much annoyance but are a real danger to the residents in the house. This swamp had not completely dried up on December 27, when I examined it, although there had been no rain for two months previously. Many mosquitoes were still breeding in the puddles; it would appear that even during the dry season mosquitoes infest the district, and in fact I had no difficulty in obtaining them for examination. The natives collected very many for me from their huts, and these for the most part belonged to the *Anopheles* genus. About a mile away from the Cape, on the Coast, is another promontory jutting out into the sea. This presents an ideal site for a Sanatorium, as the land at the back is high and level for a considerable distance stretching into the interior, and also no native huts appear within a radius of a mile.

I took the opportunity of travelling with Lieutenant Young to see some of the stations up the river. Unfortunately only a short time was at my disposal, so that no detail work could be done. We travelled up river in December, when the dry season was fully established. At McCarthy Island, one hundred and fifty-three miles up, is the most important station; here there is a Government House and a few traders' factories. The island is very low-lying and swampy; a rice swamp, at this time dried up, lies by the edge of the river. Close to the principal native

town, and behind, the town, half-a-mile away, there is also another large swamp running along the centre of the island, probably the old course of a river. In the large swamps innumerable mosquitoes occurred. Government House is situated close to the native town, surrounded by old stone ruins, which afford suitable hiding-places for mosquitoes in the dry season ; there were many mosquitoes about. One European was staying in the Government House when we visited it, and in his room I found, in the middle of the day, innumerable specimens of *A. funestus* and its varieties resting on the walls. A well present in the compound was devoid of *Anopheles* larvae, though larvae of the *Culex* type were present in numbers. These mosquitoes must have either flown from the swamp at the back of the town, where I found larvae in amongst the grass, or must have been bred in the river ; here I failed to detect larvae ; small fish occurred in the crevices and bays of the bank. The native town is similar to Bathurst, trenches in the streets drain into the river, and in the wet season, in consequence, innumerable breeding-places for mosquitoes are formed. Water is obtained by the natives from the river, as there are no wells. McCarthy Island appears to me to be quite unsuitable to permit of preventative operations against mosquitoes. One must rely on such means as segregation and personal precautions against these insects. This latter method of precaution was, I believe, very successful in the case of Mr. Barnois, who, in 1898, stayed at McCarthy Island for some time without taking malaria fever, a mosquito net being successfully used.

A native Mandingo town, Baia, was visited, and the country round explored. This town was situated two miles away from the river. The country round was perfectly level, most of it being cultivated ; ground-nuts, cuscus, and a bean being the chief products. Everywhere the ground was dried up within a radius of two miles ; there were no breeding-places for mosquitoes within this radius, except one very deep well, forty feet, which had been discarded ; in this well only the *Culex* type of larvae occurred. In the huts innumerable quantities of *Anopheles funestus* and its varieties occurred, which could be obtained at any time of the day off the walls. So completely was there an absence of water that, at the margin of a well from which water was constantly being drawn, bees, butterflies, and other insects were present in great numbers, drinking up the water spilt on the ground. At this time of the year the only breeding-places for mosquitoes are in the neighbourhood of the swamps two miles away. Lieut. Young informed me while out shooting that he came across a tub in some fields a short distance from the town in which mosquito larvae were present. I searched along the margin of these swamps, but I failed to obtain larvae here or in the pools dug out for cattle.

III. ENDEMIC MALARIA IN THE GAMBIA

The discovery, both by Professor Koch in German East Indies, and by the members of the Royal Society Malaria Commission in West Africa (1900), of the prevalence of malarial parasites in the blood of native children in an endemic area up to twelve years of age has given us a method by which a correct estimation of the presence and the extent of malaria in a district can be ascertained by the systematic examination of the blood of the children.

While in the Gambia I obtained some one hundred and thirteen children from 0 to 15 years of age for examination ; the children were chiefly from the town of Bathurst and a native town seven miles away at Cape St. Mary ; a few were also available for examination at a small Mandingo town, Baia, one hundred and eighty-five miles up the river. At Bathurst the children examined were those who came to the out-patient department to be treated for various ailments, or were brought by their mothers, who had come themselves for treatment. Fourteen in all were obtained in this way, only one of them showing signs of fever (101·8°) at the time of examination ; some were treated for worms, bronchitis, or injuries. The remainder of the children examined were those who came to be vaccinated at the house of the Public Vaccinator, Dr. Taylor ; they were, to all appearance, in good health. At the Cape and Baia the children were brought to me by their parents. The blood of many of the children at Bathurst was examined in the fresh condition, as well as by means of smears made in the usual way on a glass slide ; all the smears were stained by a modification of Romanowsky's method, recommended to me by Dr. MacConkey.

From the table (No. I) given below it will be seen that **eighty per cent. of the children examined harboured malaria parasites in their blood,** and these occurred in children practically equally up to ten years of age (the numbers are too small to give correct percentages at the various age periods). After ten years of age, out of thirteen cases examined seven were found infected ; in these cases the malaria parasites were rare in the blood ; in fact, in all except in one case, the parasites encountered on the films were very few.

TABLE I

Shewing Number of Children in the Gambia Examined and found Infected with Malaria Parasites

Age	No. Examined	No. Infected	Percentage Infected
0-1	18	13	—
1-2	13	11	—
2-3	13	11	—
3-4	17	15	—
4-5	17	14	—
5-6	9	8	—
6-7	4	4	—
7-8	5	4	—
8-9	2	2	—
9-10	2	2	—
10+	13	7	—
	113	91	80
0-5	78	64	82
5-10	22	20	91
10-15	13	7	53·8

Time and opportunity did not permit of the collection of a larger series of cases; it was found especially difficult to obtain children for examination in country districts; still it will be seen that the malaria index in Bathurst is very high, and consequently the chance of infection for the new-comer under suitable conditions is also very great.

It is of interest to note the high percentage of quartan parasites (*Haemamoeba malariae*) among the cases examined, viz., 31·8 per cent., and also the large percentage of crescents (*gametocytes*) observed in the blood, viz., 32·3 per cent., in those cases in which the aestivo-autumnal fever parasite (*Haemamoeba praecox*) occurred. I believe this large percentage has not been observed before on the Coast.

The parasite of tertian fever (*Haemamoeba vivax*) was only found in three cases.

The children infected at Bathurst came from all parts of the town, and no indication was obtained from the examination of their blood that malaria was less prevalent in one street than in another; considering the small size and the equal distribution of suitable breeding-places for the malaria-carrying mosquitoes throughout the town, it is not to be expected that at any one part the malaria index would vary. Table II gives the results of the child examination in the Gambia.

TABLE II

Shewing the Number Infected and Nature of Infection in Children of the Gambia

Children at Bathurst—

Age	No. Examined	No. Infected	Nature of Infection. Remarks
0-1	15	11	(1) Age ten months, blood very watery, aestivo-autumnal ring forms, crescents (in fresh preparation flagellation took place rapidly), pigmented mononuclear leucocytes (2) Age three months, aestivo-autumnal ring forms, pigmented mononuclear leucocytes (3) Age eight months, small aestivo-autumnal ring forms, few crescents (4) Age six months, large and small quartan forms, pigmented mononuclear leucocytes (5) Age two months, many aestivo-autumnal ring forms, one crescent, pigmented mononuclear leucocytes (6) Age seven months, many aestivo-autumnal ring forms, crescents, pigmented mononuclear leucocytes (7) Age eleven months, aestivo-autumnal ring forms (8) Age eight months, few aestivo-autumnal ring forms, one crescent (9) Age ten months, aestivo-autumnal ring forms (10) Age eight months, aestivo-autumnal ring forms, pigmented mononuclear leucocytes (11) Age eight months, many ring forms, double infection of corpuscles, pigmented mononuclear leucocytes Three of the children not infected were under three months old.
1-2	11	9	(1) Many aestivo-autumnal ring forms, pigmented mononuclear leucocytes (2) Ditto (3) Ditto (4) Ring forms, large quartan parasites, flagellating forms in fresh slides, in stained slides many quartan gametocytes, pigmented mononuclear leucocytes (5) Aestivo-autumnal ring forms, pigmented mononuclear leucocytes (6) Ditto (7) Quartan parasites all stages, few gametocytes, pigmented mononuclear leucocytes (8) Aestivo-autumnal rings, numerous pigmented mononuclear leucocytes (9) Few aestivo-autumnal ring forms, numerous pigmented mononuclear leucocytes
2-3	5	4	(1) All stages of quartan (2) Aestivo-autumnal ring forms (3) Ring forms, large forms quartan (4) Very numerous aestivo-autumnal ring forms, pigmented mononuclear leucocytes. This child was an Assyrian; had fever two weeks ago; temperature was 97·4 on examination

Children at Bathurst—continued—

Age	No. Examined	No. Infected	Nature of Infection. Remarks
3-4	5	5	(1) One crescent present (2) Ring forms, large quartan parasite, one crescent (3) Few aestivo-autumnal ring forms (4) Many aestivo-autumnal ring forms (5) Few aestivo-autumnal ring forms, twelve crescents, which had not changed after two hours, were counted in drop of blood, others had flagellated very soon after the blood was drawn
4-5	9	7	(1) Three crescents in slide (2) Large and small quartan forms, pigmented mononuclear leucocytes (3) Few aestivo-autumnal ring forms, pigmented mono-nuclear leucocytes (4) Ditto (5) Ditto (6) Ring forms, half-grown quartan parasites, pigmented mononuclear leucocytes (7) Large quartan forms, pigmented mononuclear leucocytes
5-6	1	0	
6-7	3	3	(1) All stages of quartan parasite, pigmented mononuclear leucocytes (2) Quartan parasites, pigmented mononuclear leucocytes (3) Aestivo-autumnal ring forms, one crescent
7-8	2	1	(1) Few aestivo-autumnal ring forms, one crescent, pigmented mononuclear leucocytes
8-9	0	0	
9-10	2	2	(1) Few small and half-grown tertian parasites, tertian gametocytes (2) All stages of quartan parasites
10 +	5	2	(1) Age ten years, only one aestivo-autumnal ring form present in slide (2) Age eleven years, four aestivo-autumnal ring forms present in slide. The ages of the non-infected children were fifteen, fourteen, fifteen years, respectively

Children of Baia—

Age	No. Examined	No. Infected	Nature of Infection. REMARKS
2-3	3	3	(1) Many aestivo-autumnal parasites (2) Ditto (3) Ditto
3-4	1	1	(1) Few aestivo-autumnal parasites
4-5	1	1	(1) Pigmented mononuclear leucocytes, few aestivo-autumnal parasites
5-6	1	1	(1) Large tertian parasites
7-8	1	1	(1) Few aestivo-autumnal ring forms
10+	3	2	(1) Few aestivo-autumnal ring forms (2) Ditto
	10	9	

Children at the Cape—

Age	No. Examined	No. Infected	Nature of Infection. Remarks
0-1	3	2	(1) A few aestivo-autumnal ring forms, two crescents (2) Ring forms, large quartan parasites
1-2	2	2	(1) Ring forms, large quartan forms, quartan gametocytes (2) Three crescents, ring forms, large quartan forms, quartan sporocytes, pigmented mononuclear leucocytes
2-3	5	4	(1) Few aestivo-autumnal ring forms (2) All stages of quartan parasites (3) Aestivo-autumnal ring forms, one crescent (4) Sporocytes, gametocytes, and small forms of quartan
3-4	11	9	(1) Ring forms, sporocytes and gametocytes of quartan (2) Aestivo-autumnal rings (3) Few aestivo-autumnal rings (4) Quartan gametocytes (5) Few aestivo-autumnal ring forms, few crescents (6) Very few small quartan forms, quartan gamecocytes (7) Ring forms, few half-grown quartan forms (8) Few aestivo-autumnal ring forms. In this slide three trypanosomes were present[1] (9) Aestivo-autumnal ring forms, one crescent
4-5	7	6	(1) Few aestivo-autumnal parasites (2) Ring forms, half-grown quartan parasites (3) Small quartan parasites (4) Small quartan form, quartan gametocytes (5) Quartan gametocytes and small forms (6) Aestivo-autumnal ring forms
5-6	7	7	(1) Few aestivo-autumnal ring forms (2) Many aestivo-autumnal ring forms, few crescents (3) Few aestivo-autumnal ring forms, one crescent (4) Few aestivo-autumnal ring forms (5) Ditto (6) Quartan gametocytes and small forms (7) Aestivo-autumnal ring forms
6-7	1	1	(1) A few tertian parasites
7-8	2	2	(1) Aestivo-autumnal ring forms (2) Ditto, few crescents
8-9	2	2	(1) Many quartan gametocytes, few ring forms (2) Few aestivo-autumnal ring forms
9-10	0	0	
10+	5	3	(1) Very few aestivo-autumnal ring forms (2) A few half-grown quartan parasites (3) Ring forms, large quartan forms, and quartan sporocytes
	45	38	

1. See Dutton, *Preliminary Note upon a Trypanosome in the Blood of Man. Thompson Yates Laboratory Reports*, vol. IV., pt. II. Liverpool, 1902.

Presence of the Malaria Parasite in its Definitive Host

Mosquitoes of the *Anopheles* genus were collected for examination from the soldiers' quarters and brought to me by an orderly from time to time in small batches; these were kept from one to three days, to allow of the digestion of the last meal of blood, and then dissected. Out of twenty-seven complete dissections, three mosquitoes were found infected; two of these had zygotes in the stomach wall, three in one and two in the other, from six to ten days old, variety undetermined. In the other infected mosquito, sporozoids were found in the salivary glands, principally in the middle lobes. Out of a dozen mosquitoes (*Anopheles*) caught in the hospital, chiefly from the West Indian soldiers' ward, two were infected. A few zygotes about three days old were found in the stomach in both cases, and were identified as *Haemamoeba malariae*; these two mosquitoes were caught in the dispenser's (native) net three days previously. An examination of his blood was made, but no parasites were detected; he had previously had no symptoms of malaria. It is very probable that these mosquitoes were infected in the town, and not from the soldiers in the barracks or hospital. To obtain *Anopheles* from these places it was necessary to visit the barracks just as dawn appeared, 6 a.m., when they were found on the walls, just over the beds of each soldier; during the day I was never able to detect an *Anopheles* mosquito in this room; they were probably all driven out by the disturbance of rolling up kit, etc., in the morning. The *Anopheles* examined were all *Anopheles costalis*, except three *Anopheles pharoensis*, which were negative.

At the Cape the natives brought me a good number of mosquitoes collected from their huts; nearly all collected in this way were of the *Anopheles* genera, including *Anopheles costalis* and *Anopheles funestus*, the number of the latter greatly predominating.

Twenty-four complete dissections were made, two *Anopheles funestus* being found infected; in one the salivary gland contained sporozoids in large quantities, and in the other four medium-sized zygotes with three empty capsules were present on the stomach wall. Unfortunately, many mosquitoes brought back to Bathurst had died on the way, and were unfit for dissection on arrival.

Other Haemamoebae.—The nature of the expedition did not permit of any extensive investigation on the distribution of other *Haemamoebidae* in the Gambia, still a few animals were examined.

The common yellow and green African canary was universally infected with *Haemamoeba danilewskii* (Halteridium); some tame pigeons from the various factories also showed this parasite in their blood, and also a few birds shot in the bush.

Haemamoeba relicta (Prateosoma) was never found; but the number of birds examined was not sufficient to form an opinion as to the absence of this parasite from the Gambia.

c

IV. BREEDING-PLACES OF MOSQUITOES IN BATHURST

The various breeding-places of mosquitoes which occur in Bathurst can be very suitably grouped under two heads, namely, natural and artificial. The latter are by far the most numerous and important, as many of them occur throughout the wet and dry season, while for at least six months of the year the natural breeding-places have ceased to exist. The following are the chief artificial breeding-places of mosquitoes in Bathurst:—

1.—*Canoes, boats, lighters, and cutters on the foreshore.* The making of boats appears to be a rather extensive industry amongst the natives at Bathurst ; the boats are constructed or repaired on the foreshore, in front of Wellington Street. There is always a large number of these boats lying on the beach ; some of them have been found unfit for repairs and are discarded, others are waiting their turn, it may be, for some weeks, and still others are present waiting to be launched. These canoes and boats collect rain-water, and in the larger boats the amount of water present is very considerable, and often lasts not only between the showers but for a long time after the rains have ceased. In one old boat I found water present four weeks after the last shower in October. In many of the boats the water was from one to two feet deep. On examination, as would naturally be expected, I found these boats almost universally infested with mosquito larvae. **From a rough estimation I made I calculated that each of these boats would produce two thousand mosquitoes per week ; fifty boats of all kinds producing this quantity of mosquitoes is a total of one hundred thousand insects per week distributed into the town.** It will be seen, therefore, that these boats provide excellent and very extensive breeding-places for mosquitoes throughout the wet season and for a portion of the dry. There is no doubt that the houses in Wellington Street derive most of their mosquitoes from these artificial breeding-grounds. The mosquitoes found breeding in these boats were, in order of frequency :—

Stegomyia fasciata
Anopheles costalis
Culex duttoni
Stegomyia albocephala
Culex tigripens

2.—*The Street Drains.* The street drains carrying off surface water have been described as running down the centres of the streets. They can conveniently be arranged under three heads with regard to their capacity for acting as suitable breeding-places for mosquitoes.

(*a*) The large main channels (three to six feet deep by six feet wide), which open into the river at the sluice gates. These drains occur in Hill Street, Alglesea Street, Picton Street, Blucher Street, and the large drain on one

Llewellyn Street drain, near Clifton Road, in the dry season. This drain, being constructed in sand, with no protection against falling in of the sides, allows of the formation of suitable puddles for breeding mosquitoes. *Anopheles costalis* principally breed in this drain. Water collects underneath the low bridges crossing the drain, in which many mosquitoes are found at the end of the wet season.

side of McCarthy's Square. In these drains I was never able to find mosquito larvae during the months of October, November, and the first part of December, although surface water from one-half to three feet deep was always present in them. There is no doubt that the absence of larvae in these drains was due to the large quantities of small fish which are continually swimming up and down the channels in small shoals of from thirty to sixty. The sides of the drains being perfectly smooth, no protection is afforded to the larvae. As the dry weather sets in the water gradually sinks lower and lower in the drains, until water is only present for a distance of twelve to fifteen yards from the sluice gates. This amount of water is present probably throughout the dry season, as it is tidal water which oozes into the drains through the sluice gates. In the process of drying-up, the fish die out or are killed when the drains are cleaned at the end of the wet season. The disappearance of the fish was followed by the presence of mosquito larvae in the water. At the end of December and in January I found innumerable quantities of mosquito larvae in the water in Hill Street drain, near the sluice gates, later, in Alglesea Street drain, and, just before I came away, in Picton Street and Blucher Street drains. These mosquitoes were principally *Culex thalassios*, *Culex duttoni*, and a few *Anopheles costalis*.

(*b*) Shallower drains, made of brick or stone, either partly cemented or not at all, varying from one to one-and-a-half feet deep; these drains either discharge at right angles to the larger drains or are continuations of them into the centre of the town. They vary with regard to their fitness to act as breeding-places of mosquitoes according to the seasons of the year. In the wet months they are probably free from mosquito larvae, owing to the amount of water passing along them, and thus the small fish would be able to swim up from the main drain. At the beginning of the rains and end of the wet season mosquitoes can breed in them. In October and November I found that after the rain small pools of water collected, owing to the unevenness of the cemented bed of the drain, after the main mass of water had passed away. This condition occurs in the drains of the following streets: Lemon, Hagan, and the town end of Blucher and Picton Streets. The other condition present in these drains, and of more importance than the irregularities in the bed of the drains, is the fact that many of these shallow drains are not completely lined with cement, so that they allow the ground water to percolate into them. This water I found formed a very slow stream along the course of the drain, about one-half to one inch in depth, and was very

suitable for the development of mosquito larvae, which were present here in fair numbers, chiefly larvae of *Anopheles costalis*. This condition obtained, when I arrived in Bathurst, and lasted through the months of October and November, that is, until the level of the ground water had sunk below the bed of the drain. The streets in which these conditions occurred are Dobson Street, Hagan Street, Blucher Street, Hill Street, part of Lemon and Buckle Streets.

(*c*) The other kind of drain occurring in Bathurst is that which has been described as a trench dug along the centre of the streets. These drains either communicate with the big drains or discharge directly into the swamps. It was pointed out that the depth of these drains vary, and that they often are converted into a series of pools by rubbish and sand either thrown into them or by natives walking across. I found these drains to be almost universally infested with mosquito larvae, which occurred in great quantities along their course, and were for the most part all of the genus *Anopheles*; only very occasionally did I find the *Culex* type of larvae in any amount. The small fish, which have been mentioned as being so beneficial in keeping the larger-made drains free from mosquito larvae, though occurring also in many of the grass-choked drains, are here at a disadvantage, the larvae being to a great extent protected by the grass and sticks amongst which they can hide; also pools cut off from the main masses of water occur in which fish cannot gain entrance. **These drains supply Bathurst with the majority of its mosquitoes during the months of September, October, and November.** They occur in the following streets: Fitzgerald Street, the drain runs along the centre of this street from the corner of Kent Street to Box Bar, where it is supposed to empty itself by a channel running down Lovel Place, entering the main drain leading into a swamp. The uselessness of these stretches as a whole are well illustrated by this drain, where I found the outlet completely blocked up with sand and rubbish, so that water would of necessity flow over Lovel Street to get into the swamp, and small fish for this reason also could not possibly gain entrance. Further, the drain itself had been converted into a series of long pools by the natives walking across, carrying with them sand, etc., and, lastly, the thick, rank grass growing in the bed of the drain, together with its naturally small fall, tends to retain the water in it. This drain is bricked across by two or three low, brick arches at the street crossings. Underneath these arches the bed of the drain dips, so that water collects in considerable quantity, which I found contained many *Anopheles* larvae; also, in the course of

One of the central grass-clogged drains at Bathurst in the dry season at a spot where the course of the drain is obliterated by reason of natives walking across; at the end of the wet season this drain is a series of long puddles breeding large quantities of *Anopheles*.

the drain a cemented catch-pit occurs, one foot in depth; this I also found afforded an excellent place for mosquito larvae. A few other drains in a similar condition to the one described above must be specially mentioned. Commencing at Government House and running behind the Hospital and Telegraph Station is a rather deep drain, which takes the water from a small swamp in this region, discharging into Box Bar by two smaller drains crossing Clifton Road and running through Portuguese Town. In these drains mosquito larvae are very abundant until the end of November, and especially was this the case in the two smaller drains. One of these small drains crosses Clifton Road at the back of the Hospital; being dug out of pure sand its bed naturally was dammed up by the falling in of the sides of the drain. In the dry season for this reason also the drain becomes practically obliterated. The other drains crossing Clifton Road, nearer the Cemetery, were also converted into a series of pools by the falling in of sand and rubbish. On my arrival, and up to the end of November, these two drains swarmed with mosquito larvae; from a rough estimation I made during these months I calculated that one larva was present in every four square inches of surface water. These two drains were five hundred feet long, and the water in them on an average was two feet across. From these data one is able to calculate approximately the number of larvae occurring in the drain, and, also, the number of mosquitoes issuing into the town per week from them.

The following are some of the principal streets in which the drains occur:—

Long Street, Grant Street, New Street, Allan Street, Kent Street, Clarkson Street, Dobson Street, Perseverance Street, Prometheus Street, the shore end of Buckle Street, Lemon Street, Picton Street, the town end of Lancaster Street, the swamp end of Hill Street.

In some of these streets the earth dug out to form the drain has been piled up on either side of it, so that the surface of the street falls away from the drain. These grass-overgrown ditches also occur around some of the native compounds in New Town, on the south side of Box Bar, on three sides of McCarthy's Square, and on either side of Clifton Road, bordering Portuguese Town. These drains are certainly to be condemned; even after heavy rains they appear to allow very little of the surface water to pass along them. Dr. Forde informed me that he has observed very little current in these drains after a heavy shower, and I have made a similar observation. They then collect the rain-water, which stagnates and becomes foul, and only disappears when the level of the ground water sinks below the bed of the ground, that is about two months after the rains. They have been shown to be often

converted into a series of pools by the pouring in of sand, and are often blocked at their outlets in a similar manner, so that fish cannot gain entrance. They thus form excellent breeding-grounds for mosquitoes, and, as I have shown, especially for the malaria-bearing variety (*Anopheles*). The species of mosquitoes found in the drains of Bathurst in order of frequency are :—

Anopheles costalis.
Culex thalassios (in drains containing tidal water).
Culex hirsutipalpis.
Culex duttoni.

3.—*Wells.* During the month of October I found that the wells of Bathurst were not a fruitful source for mosquitoes, though larvae were present in some of them ; still, as a whole, they did not provide extensive breeding-places. After the rains had ceased and as less and less water was to be found in the drains and swamps I observed that mosquito larvae occurred more frequently in the wells. The public wells, some fourteen in all, were examined at various times during October, November, December, and January ; in only one (Lancaster Street) were mosquito larvae found. This well had become foul, chiefly from rubbish thrown into it, and very soon after mosquito larvae swarmed in the water. The native population almost universally obtained drinking water from the public wells, and water is thus being drawn from these wells practically all day long. It would appear from this constant disturbance of the water that these wells were not suitable breeding-places, as I found that in many similar wells in private compounds in which the water was equally good, though less frequently drawn, mosquito larvae were easily obtained. Fifty-five large private wells were examined, occurring in compounds throughout the town ; it was found that those wells which had good covers were almost free from mosquito larvae, while all the others contained larvae in quantities depending on the frequency with which water was drawn from them. The small shallow tub wells occurring in large quantities all over the town were found to contain mosquito larvae, chiefly of the *Culex* kind, in sixty per cent. of those examined during the month of October. It was also noticed that when one of these wells became foul, and from this cause discarded, the larvae occurred in greater abundance ; not a few such tub wells exist throughout the town. It was also noticed how very rapidly the larvae sank to the bottom of the well on the slightest disturbance of the water. All the wells, both public and private, were re-examined systematically in the latter part of December and January by the Sanitary Inspector with the object of determining the percentage in which mosquito larvae occurred. The result is given in the following table up to the time of my departure from Bathurst ; this includes nearly all the public and private wells, and probably about two-thirds of the small tub wells.

	No. Examined	No. of Wells covered	Presence of *Anopheles* larvae	*Anopheles* and *Culex* type of larvae	*Culex* type of larvae
Tub wells ...	217	—	—	8	198
Stone wells—					
Public ...	14	1	—	1	—
Private ...	55	13	1	8	35

From the above table it will be seen that over ninety per cent. of the small tub wells already examined were breeding mosquitoes. With regard to the large private wells those in which larvae were found were generally the ones improperly covered. In this connexion it is interesting to record the examination of a large stone well in the yard at the Police Station, which was extremely well covered in, the only entrance as far as one could detect on superficial examination was the small wire gauze lid. In the early part of my stay in Bathurst I had not detected any mosquitoes in the water, in December this well became foul ; it was emptied and cleaned, but afterwards the water was worse than ever, having a very strong smell ; the contamination was very probably due to a leaking midden in the neighbourhood. Orders were then given that no water was to be taken from the well, and the small lid was locked. Before the filling in of this well was accomplished I examined this well and found mosquito larvae were present in it, and many young mosquitoes dead on the surface of the water. On closer examination of the well I discovered two small holes between the stone rim and wooden cover. These two holes were the only means by which mosquitoes could gain entrance into the well to lay their eggs, but the young mosquitoes apparently had failed to detect these exits and had died in their endeavours to get out through the wire gauze of the lid in the cover. It will be seen how important it is for wells to have a perfectly fitting cover. The mosquitoes which breed in the wells at Bathurst during the latter part of the year are :

Anopheles costalis (chiefly in the large built wells)
Culex fatigans
Stegomyia fasciata
Culex tigripes
Culex duttoni

Breeding-Places Occurring Round Houses and in Compounds

The number of mosquito breeding-places present in compounds was found to vary with the social position of the occupier. In small compounds of the poorer natives, where one or two huts were present, no breeding-places were found. These natives had no discarded bottles, etc., in which water could collect, nor were wells or tubs or any article for the storage of water present, sufficient water for the day being drawn from one of the public wells. These compounds were exceedingly clean and tidy, and no mosquitoes were found breeding in them. Excepting these, breeding-places were found and increased in extent and number in proportion to the wealth and position of the occupier of the compound, reaching a maximum on the premises of the larger traders (natives and white), where innumerable facilities for the development of mosquitoes were afforded. These breeding-places included all those domestic articles which are capable of containing a small quantity of water after showers lasting over a week without being dried up, or are not dried up between the frequent showers in the wet season. Such articles found were broken bottles, either stuck on a wall or scattered over the compounds, iron pots, old calabashes, tin-lined packing cases, cocoanut husks, fowl troughs, and old tins of all sorts. There was found an extraordinary amount of such-like rubbish in some of the factory compounds, the more specialized breeding-places included tubs, used for the storage of rain-water or as wash tubs for bottles, or in which water was placed for the preservation of the tub. Large barrels in which fibres were soaked, garden tubs in which water was stored for gardening purposes, old iron boilers for the collection of rain-water, improperly covered rain-tanks formed other breeding-places. In some of the factories a small gutter six inches across by four feet deep is let into the cemented floor of the yard around the ground-nut store house. This gutter is kept full of water to prevent the entrance of the ground-nut insect into the store. These gutters swarmed with mosquito larvae. In some yards a small channel runs down the centre to drain off rain-water, and is generally covered over with a board. It was found that some of these had become clogged up at intervals with sand and rubbish, so that small pools of water collected along their course; these pools acted as breeding-places for mosquitoes. **An account of an examination of one of the larger European factories will illustrate to what extent mosquitoes are bred by the white man in the tropics on his own premises. In the factory yard were six barrels containing water, in some the water was very foul; in the garden were seventeen tubs containing water for gardening purposes, and besides this number of tubs there were eight small wells, all uncovered. In all these articles mosquito larvae were present; in**

the barrels in the yard the water swarmed with *Culex* and *Stegomyia* larvae, and in the wells and tubs in the garden the larvae of *Anopheles* and *Culex* were found in all of them in good numbers. Besides these breeding-places there were many domestic articles scattered about in odd corners of the yard, which in the wet season would also have acted as breeding-places.

The Species of Mosquitoes Found in Compounds

It was observed that larvae of *A. costalis* were frequently found in rain-tubs and smaller articles containing water. Though many of these larvae may have been originally transferred to some of these articles along with the water drawn from the well, yet the occurrence of batches of larvae of the same age and in fair numbers would tend to show that this species of mosquito avails itself of these small collections of water in which to breed.

Stegomyia fasciata, in tubs and old bottles, etc.
Culex fatigans „ „ especially when the water was foul
A. costalis, tubs and barrels
Culex duttoni
Culex hirsutipalpis
Stegomyia pagens (rare), in ground-nut gutters

The wash-tubs, garden-tubs, wells, and rain-barrels occurring in compounds form the chief source of mosquito in Bathurst for at least six months of the dry season, when all other breeding-places, artificial and natural, have ceased to exist.

Natural Breeding-Places for Mosquitoes in Bathurst

The natural breeding-places for mosquitoes in Bathurst occur for the most part in the swampy districts, namely, Box Bar, Half Die, and the small swamp behind the Hospital; a few natural hollows in some of the streets, more especially those bordering on the swamps, collect rain-water, in which mosquitoes breed.

Box Bar. In the wet season this swamp is covered by a considerable mass of water, which encroaches on to New Town and Portuguese Town and into the centre of Bathurst at Albion Place, the streets in these neighbourhoods being flooded at this season of the year; how far this swamp acts as a breeding-place for mosquitoes in the height of the wet season (July and August) I cannot say definitely, but judging

from the conditions which obtain at the end of the wet season, when I personally examined the swamp, I surmised that puddles are formed in the natural hollows of the streets all along the margins of the swamp, namely, Albion Place, Perseverance Street, etc.; indeed I found, on arriving at Bathurst, remains of puddles in these streets; some were dried up, others still contained a little water, in which *Anopheles* larvae were present. Box Bar swamp presented some interesting features during the months of October and November. A long, partially constructed central channel runs the length of the swamp, opening by means of double sluice gates into the Oyster Creek at the back of the town. This channel contains a large quantity of water, which overflows on to the swamp after heavy rains. On either side of this channel the land gradually rises one to three feet to Portuguese Town on the one side and New Town on the other; it is covered with grass and a few low bushes. This land has a very uneven surface; there are very many hollows and depressions occurring at intervals amongst the grass, and also not a few large round holes three to four yards across, which may have been formed by the natives in obtaining sand; also, on the borders of the swamp, especially around New Town, trenches occur choked with grass. Lastly, innumerable crab holes are present everywhere, and on to the surface old tins and calabashes are thrown. In the central mass of water I was never able to obtain mosquito larvae, undoubtedly owing to the enormous quantities of small fish which are always present in the water. But in October and November the hollows and depressions mentioned above as occurring amongst the grass contained water, which was cut off from the main channel, and thus unable to drain away, nor could small fish gain entrance; it was thus to be expected that these pools should contain innumerable mosquito larvae. Three large pools in close relation to this swamp, situated together near the cemetery, must be specially mentioned; these pools measured twenty to twenty-five feet across, and contained water in December, when the swamp pools proper had completely dried up; they were used then for watering cattle. As Box Bar swamp dried up these pools became more and more infested with mosquito larvae, though one of them remained free for some time. In it the water was comparatively sweeter than the other two, and small fish were present. As the dry season advanced the water in these pools became exceedingly foul, and enormous numbers of larvae, chiefly *Anopheles*, were found. A long, narrow pool occurs in this neighbourhood, along the side of the cemetery; this pool communicated with the creek, and is flushed with tidal water; though the water present in it was foul, and had a distinctly unpleasant smell, no mosquitoes were found breeding in it.

Half Die. This swampy district is drained in a similar manner to Box Bar by means of a central channel opening into the creek by sluice gates. No mosquitoes were ever found breeding in the central mass of water. The swampy surface is much more level than Box Bar, and very little grass is present. Native huts are built close up to the margin of this swamp, practically on all sides, and it is in their compounds

and in the adjoining streets, in which depressions either natural or dug out by the natives occur, in which mosquitoes breed. In these compounds, also, I found many old crab holes containing larvae. Owing to the comparative evenness of the surface, pools formed in this neighbourhood cannot exist for long after the rains have ceased ; by the middle of November there were only one or two natural breeding places to be found. The level of this swamp is being rapidly raised by the deposition of sand taken from the beach ; already the area which has been completed has proved a success. The native compounds built on it were comparatively free from water during the rainy season last year. The remaining swamp to be mentioned is a small one extending from the back of Government House to just beyond the telegraph station, and is limited on the town side by Picton Road. In this swamp water collects in the rainy season and lasts for some time after the rains have ceased, especially in the drain running down its centre which has been previously described ; pools and depressions also occur amongst the grass. This swamp supplies Government House, the Hospital, and other European quarters in this neighbourhood with a considerable number of mosquitoes. The mosquitoes found breeding in the above swamps were in order of frequency : *A. costalis* (the larvae of this mosquito were found in disused crab holes containing water on several occasions, principally in Half Die swamp), *Culex halassius*, *Culex duttoni*, *Culex tigripes*, *A. pharoensis* (rare), *Culex euclastos* (rare).

The swamps described above are not flushed by tidal water owing to the low wall which surrounds the town in their neighbourhood, and also because the sluice gates are only opened at ebb tide ; still a little tidal water gains entrance into the main channel by leaking through the gates.

There is a considerable variation in the number of breeding-places in Bathurst according to the season of the year. In October, when I landed there, the rainy season was rapidly drawing to a close, only a few heavy showers occurred at the beginning of the month. Altogether there was 3·81 inches rainfall. At the beginning of this month all the breeding-places described above were present, at the end of the month practically all the natural and many of the artificial (drains and boats) were dried up. Accompanying this drying up of the breeding-places there was a slight diminution of mosquitoes in some parts of the town (Government House, Hospital, Telegraph Station), but no marked diminution in factory compounds or native quarters. From November onwards, until the commencement of the rains at the beginning of June, the only places in which mosquitoes can breed are wells, tubs, and other articles for the storage of water in compounds, in the tidal water in some of the main drains near the sluice gates, chiefly in Wellington Street, and in the three pools near the cemetery. An examination of the mangrove swamp at the back of the town was made on several occasions, but no breeding-places were found, though some puddles were present which appeared to be suitable. It is very probable that the absence of breeding-places in the mangrove swamp is due to the ebb and flow of the tide and the presence of fish.

The termination of street drain at sluice gate; in the dry season *tidal water* percolates into the drain through the sluice gate; in this water many mosquitoes breed, more especially is this the breeding place for *Culex thalassius*. Theo.

Action of Tidal Water on Mosquitoes

Experiments were undertaken to see how far mosquito larvae would thrive in tidal water; larvae of *A. costalis* of various sizes were taken and were placed in tidal water in large glass jars, and supplied with food. Various percentages of salt water taken from the beach at high tide were added, at the same time control batches of similar larvae were kept in vessels containing the water in which they were breeding. It was found that many of the young and medium-sized larvae died in six to eight hours in the jars containing seventy-five per cent. of sea water, below this percentage they remained alive.

In one experiment in which larvae were placed in undiluted tidal water, one large larva remained alive and changed into a pupa in three days after the experiment started. A garden tub in which mosquitoes had been breeding was emptied and cleaned, and sea water, taken as the tide was coming in, placed in it, the other tubs in the garden being covered with mosquito netting. In four days afterwards a batch of small *Anopheles* larvae was discovered in the water, which subsequently hatched out into adult mosquitoes (*A. costalis*) seven days later. This experiment was repeated with the result that first eggs of *Anopheles* and also *Culex* appeared in one or two days after the tidal water had been placed in the tub, and subsequently adult insects hatched out from them. **From these experiments it would thus appear that certain kinds of mosquitoes can breed in tidal water** if it is not disturbed, and subsequently when the dry season had fully set in, I found larvae in suitable tidal pools, namely, as I have already mentioned, in the drains near the sluice gates in which tidal water had soaked in through the gates. In this water, which contained 1038·5 parts of chlorine per 100,000 parts, I found a few larvae of *A. costalis* and large numbers of *Culex balassios*. On another occasion, in December, I found these mosquitoes breeding in a small hole from which shells had been taken, close to the edge of the water at the mouth of Oyster Creek. The *Culex* were subsequently hatched out from this tidal water, but the *Anopheles* larvae were nearly all infested with a fungus (not identified) which gave them a woolly appearance, and I failed to hatch out any of them. I observed *A. costalis* breeding in a similar salt-water pool during a period in which neap tides occurred. The tidal water in an arm of the central channel in Box Bar, running from the sluice gates to the cemetery, had become converted into a series of small pools by partial evaporation of the water, though at every tide some water leaks through the sluice gates into this channel, but during this period it was not sufficient to replenish this small branch drain. *Anopheles* larvae were found in great numbers in these pools. It is interesting to note, also, that at this time two of the largest pools which were situated close by, and have been described as swarming with mosquito larvae, had been filled in with sand. Samples of water taken from various parts of the town were examined for the amount of chlorine present in them, the result is given in the following table.

Sample of Water	Chlorine per 100,000 parts	Chlorine expressed as percentage of NaCl
1. Public well, New Town, no mosquitoes present in water	161·2	·265
2. Public well, Lancaster Street, water rather foul, *Anopheles* larvae present in numbers	1148·0	1·89
3. Public well in Clifton Road, behind Hospital, no larvae present	186·0	0·3
4. Water (tidal) found in drain near sluice gate, Blucher Street, *A. costalis* and large quantities of *C. halassios* present	1038·5	1·71
5. Small well in private garden, *Culex* present, few only...	27·9	0·04
6. Small well in native compound, Victoria Street, *Culex* larvae present	1145·0	1·88

Mosquitoes Present in the Gambia

The commonest mosquitoes met with in Bathurst were *Stegomyia fasciata* and *A. costalis*. After these may be mentioned *Culex fatigans*, *Culex duttoni*, *Culex halassios*; the latter, a new species, was especially frequent in December and January, and bred in the tidal pools of water as described above. While in Bathurst I never saw specimens of *A. funestus*. Out of a series of *Anopheles* which had been bred, or captured in various parts of the town, Mr. Theobald has failed to detect one; on the other hand, at Baia and McCarthy Island, in the Hinterland, I did not obtain a single *A. costalis*; here all the *Anopheles* caught were the small *A. funestus* and its varieties. At Baia, out of two hundred mosquitoes brought to me by natives, all were *A. funestus* except one; these mosquitoes could be caught at any time of the day in the native huts, they were found resting on the wall, some had evidently fed not many hours previously; the ovaries were in all stages of development. At McCarthy Island I found, at 1 p.m., a great many *A. funestus* on the walls of a room in Government House in which a European slept. No other kind of mosquito was present. At Baia and McCarthy Island, in December, when everything is dried up, the river and a few marshes were the only available breeding-places. At Baia the river was three miles away from the town, and the only marsh near was two miles away. The only breeding-place in the native town was an old disused well, which was found to be thirty-four feet deep; at this depth only *Culex* larvae were found in the water. At McCarthy Island I never found mosquito larvae in the river, but in a swamp half-a-mile away from the town they occurred in great numbers. At the Cape, where a similar marsh to the one on McCarthy Island occurred, *A. funestus* was the chief mosquito found in the native town; but here *A. costalis* was also present in

small quantities. The specimens obtained were caught chiefly in the European compounds. At the Cape a few artificial breeding-places were found in compounds. It would appear then that *A. funestus* and its varieties are rural mosquitoes, and require rather special breeding-places, while *A. costalis* is essentially a town-bred mosquito, and capable of utilizing any small collections of water for breeding purposes. At Bathurst I obtained one single specimen of the genus *Panoplites*, which was caught in the prison (*P. uniformis*, Theobald). This species of mosquito was never found breeding around Bathurst, and these mosquitoes were only seen in the marshes at McCarthy Island and Baia. Here they occurred in considerable numbers, and attacked natives and whites crossing the marsh at all times of the day in a very vicious manner. The observation of Durham and others with regard to *Stegomyia fasciata* was fully confirmed at Bathurst; these mosquitoes only bite during the day, more especially in the early part of the afternoon. None of this species were collected in mosquito nets during the night. As yet we have no method by which any approach to the exact estimation of the number of mosquitoes in a district can be ascertained, although a rough estimation may be got from the number and extent of the breeding-places; also the presence of mosquitoes at times when no breeding-places can be found, can be demonstrated by the construction of artificial pools as described by the Members of the Royal Society's Malaria Commission. No reliance can be placed on the statements of the white man in Africa as to the presence, or absence, or number of mosquitoes in the district. Many men become immune to the bites of mosquitoes after a time, and on the other hand, one or two importune mosquitoes cause as much annoyance as many. With the object of obtaining some idea of the number of mosquitoes entering a house at night, I employed the mosquito net method, which was found so useful by the Members of the Liverpool Malaria Expedition to Nigeria, in detecting the presence of mosquitoes in the absence of breeding-places. The Hospital was selected as the site for the experiment, and a net free from holes was rigged up over a bed in one of the wards, in which one of the orderlies or native patients slept. The net, instead of being tucked under the mattress in the usual way, fell short of the bed clothes, a space of two to four inches being left between the edges of the bed and the bottom of the net. The net was put down every night before the sun went down. During the evening, mosquitoes obtaining entrance below after feeding would climb to the top of the net. These were collected about 6 a.m. in the morning by myself or an intelligent orderly who brought them to me. Mosquitoes failed to find exit in nets rigged up in this manner even when they have become quite lively. The result of the experiment, which is given in the following table, was carried on throughout the months of November, December, and part of January. Though this method is as yet very imperfect it might be useful and interesting to rig up a similar net in the wet season, and to compare the numbers caught at the time. During the time this experiment was

proceeding it must be remembered that all the natural breeding-places near the Hospital where completely dried up, and in the Hospital compound itself no artificial breeding-places occurred.

Mosquito Net Experiment

Date	No. present in net each day	Remarks	Date	No. present in net each day	Remarks
Nov. 1	3	1 *Culex*, 1 *A. costalis*, 1 *A. pharoensis*	Dec. 17	0	
„ 13	0		„ 18	1	*Culex*
„ 14	0		„ 19	2	*A. costalis*, *Culex*
„ 15	0		„ 20	1	*A. costalis*
„ 16	1	*Culex*	„ 21	0	
„ 17	1	*A. pharoensis*	„ 22	1	*A. costalis*
„ 18	1	*Culex*	„ 23	2	*Culex*
„ 19	3	All *Culex fatigans*	„ 24	0	
„ 20	3	*Culex*	„ 25	0	
„ 21	3	All *A. costalis*	„ 26	2	*A. costalis*
„ 22	7	3 *A. costalis*, 4 *Culex*	„ 27	7	All *A. costalis*
„ 23	1	*Anopheles*	„ 28	0	
„ 24	2	*Culex fatigans*	„ 29	4	*A. costalis*
„ 25	2	*Culex*	„ 30	3	„
„ 26	1	*A. costalis*	„ 31	1	„
„ 27 to Dec. 3		Away up river, mosquitoes not collected	Jan. 1	2	„
			„ 2	1	„
			„ 3	2	„
„ 4	0		„ 4	1	„
„ 5	0		„ 5	1	„
„ 6	0		„ 6	2	„
„ 7	3	*A. costalis*	„ 7	0	
„ 8	2	1 *A. costalis*, 1 *Culex*	„ 8	0	
„ 9	0		„ 9	0	
„ 10	0		„ 10	0	
„ 11	1	*A. costalis*	„ 11	5	1 *Culex*, 4 *A. costalis*
„ 12	0		„ 12	0	
„ 13	0		„ 13	3	*A. costalis*
„ 14	2	*A. costalis* and *C. fatigans*	„ 14	2	„
„ 15	2	*Culex*	„ 15	1	„
„ 16	2	*A. costalis*	„ 16	2	*A. costalis*, 1 *Culex*

V. PREVENTION OF MALARIAL FEVER IN THE GAMBIA—DESTRUCTION OF MOSQUITOES

Practical measures against malaria have already been briefly referred to in an earlier part of this report. It remains now to consider how far these measures are applicable to the conditions which obtain in Bathurst. For convenience we may consider them under two heads :—

1. Measures for protection of the individual—individual precautions.
2. Measures for the protection of the community.

Preventive measures, including the prophylactic use of quinine and the various methods for the prevention of bites from mosquitoes, which can be adopted by every-one in the tropics, have already been carefully described and summed up by various writers, so that little need be said here. *There is no doubt that the number of bites from the Culicidae in the tropics can be greatly diminished by the careful use of the mosquito net, and thus the risk of infection from malaria lessened. But even this simple measure, as other observers have said, is astonishingly neglected and abused in Africa, and this abuse, unfortunately, is greatest among the European traders and their clerks, where the chance of malarial infection is greatest.* It is curious to note, after very careful demonstration in the way in which the net should be used, how soon one finds the nets of the Europeans acting as mosquito traps. In Bathurst a few of the Europeans have anti-mosquito bedrooms; they are on the whole good, but great care will be necessary to keep them free from holes, as they are made of mosquito netting; also the 'boys' are very apt to leave the doors open during the day, thus allowing the entry of mosquitoes. I understand that Government will soon supply all the officials with a mosquito-room, and there is no doubt that with care they can be used with some measure of success. In this connexion it would be a great advantage if the ward at the hospital in which the European patients are treated were made mosquito proof. Mosquito nets over patients' beds are unsatisfactory, owing to the necessary disturbance which takes place in connexion with the treatment of the patient. Besides the precautions directed against mosquitoes, there is no doubt that exercise and fresh air helps one, and in Bathurst special facilities exist for such exercise. There is a fairly good road for cycling, driving, or riding; and amongst games, tennis, cricket, and even football may be played. It is probable that it will be some time before the malaria mosquito investigations will be appreciated by the white man in Africa, and even when this desired result is attained, one cannot rely upon the

individual to protect himself against malaria. Therefore an important matter presents itself for consideration in each West African Colony, namely, how far can the white population be protected and to what extent can suggested measures be made applicable to the various districts and towns in the Colony? The three most important suggestions which have been put forward for this purpose (the protection of a country) are:

1. The destruction of the malaria parasite in the intermediary host (man) by means of quinine.
2. Segregation.
3. Destruction of the mosquito.

Koch first suggested the administration of quinine on a large scale, and applied it with some success to communities in German East Indies. In Africa, at Lagos, quinine is given gratuitously to the natives. This measure to be efficient must be energetically carried out, that is, each individual member of the community harbouring malaria parasites must be dosed with the drug. Neglecting the large expense it would entail this measure alone is not applicable in Bathurst, it would be difficult to carry out in an efficient manner. It would be impossible, until education has further advanced, to get the natives to submit to such treatment without using force.

In Bathurst, the valuable method of segregation is not feasible, as the town is already laid out, and good houses, occupied by Europeans, are situated in many of the streets, and surrounded by, as a rule, equally well-built native quarters; still, in a small way, in one part of Bathurst this measure could be applied, namely, to the piece of land extending from the beach to Clifton Road, on which are situated Government House, Hospital, and other European quarters; this area should be kept free from the native huts. The method of segregation is applied to some extent among the European Commissioners, their huts at the various native towns being built three hundred yards away from the native compounds. The segregation principle should also be adopted when new administrative quarters are built in the Colony, new bungaloes should not be erected within a distance of half-a-mile from the native quarters; this rule should also be seriously considered by the European traders choosing a site for a new depôt.

The last method, which has been so ably advocated by Professor Ross, deals with the destruction of the mosquito by the elimination of its breeding-places. In this method the point aimed at is to reduce the number of all species of mosquitoes in certain suitable districts; it appears to me that the town of Bathurst is especially suitable for the accomplishment of such a measure. It is situated as described on a practically isolated piece of land surrounded on nearly all sides by a broad expanse of water. The amount of land to be dealt with is comparatively small, namely, about a square mile, the surface is fairly level and sandy, readily absorbing water. In this area the breeding-places of mosquitoes are a known quantity, the artificial (those made by man) being in excess of the natural. The rainfall for a tropical country is

very small, and rain occurs only in four out of the twelve months of the year. Finally, in the dry season, as already stated, the breeding-places of mosquitoes occur only in the various yards and compounds. Excluding malaria, there are other reasons why an attempt at the destruction of mosquitoes should be undertaken in Bathurst, the close proximity of Dakar and St. Louis in the Senegal, at which places epidemics of yellow fever have broken out from time to time, is a danger to the inhabitants of Bathurst (in 1900, at Dakar and St. Louis, there were four hundred and ninety-five cases of yellow fever, with two hundred and twenty-five deaths), thus the probability of yellow fever being introduced into Bathurst from these ports by traders and others,* and its spread amongst the Europeans in this town by the means of mosquitoes is not to be disregarded. There is yet another disease very prevalent in Bathurst which is also spread by means of mosquitoes, namely, Filariasis. An examination of the blood of a number of the inhabitants revealed the presence of filarial embryos in thirty-four per cent. of those examined. Cases of Elephantiasis are frequently seen in the streets of the town. In Bathurst it is thus especially needful to diminish as far as possible the number of mosquitoes which infest the town.

In chapter IV, I have described the various mosquito breeding-places occurring in Bathurst ; here it remains to discuss the methods most suitable for their abolition.

Artificial Breeding-Places. The discarded domestic articles, including tins, bottles, calabashes, etc., must be collected and removed from all compounds in the first instance, and provision against their re-accumulation is necessary ; this might be accomplished by a systematic collection under the supervision of the Sanitary Board. The rain tanks and barrels for the storage of rain water require well-fitting covers ; such tanks should not be allowed in compounds without anti-mosquito covers, as not only do they breed mosquitoes, but the stored water soon becomes foul by dust, dirt, and insects which collect in them. Water tubs required for the soaking of fibres should be completely emptied at least once a week, and refilled with fresh water. As a further safeguard, kerosine oil should be applied to the surface of the water when the tub is refilled. Tubs for the storage of water for gardening purposes should be limited in number, and only sufficient water for the day ought to be stored in them. These tubs should be completely emptied of water by turning them upside down every day ; if this is not done a very considerable quantity of mosquito larvae are likely to remain in the small quantity of water left in the bottom of the tub. Gutters round the ground-nut stores, when filled with water, should be treated at the same time with kerosine oil, an ounce of which would probably be sufficient for each gutter.

Boats. The following methods of dealing with boats naturally suggest themselves : old boats and canoes should not be allowed to remain on the beach, such discarded boats should be broken up. Boats awaiting repairs should be turned bottom uppermost, larger boats and hulks, staying short periods on the beach, should be carefully

* See Chapter I

inspected, the rain water collected in them should be baled out as much as possible, and, in addition, kerosine oil or other culicicide should be applied, as it is impossible to bale out all the water from such boats, and even in a very small quantity of water many mosquitoes can breed; the boats on the shore should be inspected at least once a week.

Wells. There is no doubt that in Bathurst there is an excess of small tub wells out of proportion to the requirements of the natives. In the first instance, in dealing with these wells their number should be reduced; this could be accomplished by filling in these wells as they become foul (a condition of affairs which often occurs), and prohibiting the digging of new wells without the consent of the local authority. A better plan would be to do away with the majority of the native tub wells and, in their stead, to increase the number of public wells, properly covered and under the control of the sanitary authority. Dr. FORDE has devised, as a preliminary measure to control the breeding of mosquitoes in these small wells, a very ingenious cover, which also could be utilized as a cover for rain tubs and barrels. This cover consists of a large iron hoop obtained from discarded barrels, to which is fastened all round a piece of stout calico or sacking free from holes, in such a manner that a good deal of sag is left in the material. After water is obtained from the well the hoop is thrown over the mouth, and the calico catching on the rim of the well completely closes the entrance and is kept taut by the weight of the iron hoop. This cover is so simple, and, however carelessly applied, must effectually close the entrance of the tub against mosquitoes, that I think it is well worthy of extensive use in the town. Dr. FORDE had lately informed me that these covers are now being made in Bathurst, and are sold to the natives for the sum of fourpence. Another method which I believe to be feasible, and certainly applicable to the larger private wells, is to stock them with fish; indeed, I came across four wells in Bathurst in which fish were present, and in which I could never detect mosquito larvae. The fish in these wells had been obtained from one of the large drains in the town; unfortunately, I do not know the species. With regard to the large private wells, it is very essential that these should have proper anti-mosquito-proof covers.

Street Drains. It appears to be unfeasible to allow the open street drains to act as drains for subsoil as well as surface water; in fact, many of the larger drains have been well cemented to exclude sub-soil water. From the anti-mosquito point of view it is very desirable that the central street drains should only be utilized for carrying away surface water. It has been shown that when sub-soil water percolates into these drains a most suitable condition for the breeding of mosquitoes is brought about. It is therefore necessary that the bed and sides of the street drains should be well cemented. Many of the shallow drains require attention in this respect. Catchpits in the course of the drain are useless and should be abolished, and also the dips in many of the drains occurring underneath the small bridges at the corners of the streets. Frequent brushing

PLATE IV

Raising the level of Halt Die Swamp by deposition of sand taken from the beach.

out of the drains at the close of the wet season would be helpful for the purpose of removal of the small pools of water which collect in some parts of the bed of the drain. Special care ought also to be taken to provide for the entrance of fish, which have been shown to be such excellent mosquito scavengers. The centrally situated grass-clogged drains ought to be abolished; as drains they are inefficient, and as they form the chief source of mosquitoes in Bathurst at certain times of the year, they urgently require attention; many of them could be got rid of by filling in with the earth and sand piled up on either side of these drains. Their place should be taken by superficial saucer-shaped drains. The filling in of these drains will take some little time, and it will be necessary to adopt some method in the meantime to prevent, or at least to diminish, the numerous mosquito larvae infesting them. The only feasible way will be the employment of labourers to keep the drains free from long grass and rubbish, and the intelligent application of kerosine. Also special attention should be taken to keep the mouths of these drains free from rubbish, so that small fish can gain easy entrance to them.

Natural Breeding-Places. The abolition of the breeding-places occurring in the swampy districts of Bathurst is a work which will take some time, and consists practically of raising the level of the swamps by the deposition of sand, together with a proper system of drainage. At Half Die swamp the process of filling in was being pursued with great rapidity during my stay at Bathurst, and a great portion of this area has been raised two or three feet; still there is a large area of a swampy nature in Bathurst to be treated in this manner, and until this is accomplished it will be necessary to provide some other method of ridding these districts of mosquito breeding-places. In this connexion, it must be remembered that mosquitoes do not breed in the central mass of water in the swamps, this water being disturbed by winds, the rise and fall of the tide (to some extent), and, also, it is well stocked with fish, so the mosquito breeding-places to be dealt with will be found along the borders of this water, and will vary in position according to the height of the water in the swamp.

Mention has been made of the holes, irregularities, ditches, and large pools which occur on the borders of the swamps (chiefly Box Bar swamp), and in which it was shown mosquitoes bred in great quantities. Such-like breeding-places could be dealt with by filling them up with sand—the number of them is not very great; around the borders of Box Bar, for instance, there are only about fifteen of the larger holes to be dealt with. This work could easily be accomplished during the dry season by a few labourers. During the rains special inspection should be made of the borders of the swamps, and the breeding-places found treated with kerosine or other oil; I believe that the application of kerosine in an intelligent manner would produce some good results, nor do I think the cost would be very great. In the streets bordering on the swamps, holes and irregularities occur, but more especially are they found in the native compounds in these situations, and in the rainy season, when the swamp water is high,

breed many mosquitoes. Such streets and compounds urgently require attention, and I would specially mention in this connexion Albion Place, Prescot Street, Perseverance Street, and other streets around Box Bar. The natives themselves in these districts could do a great deal by raising the level of their compounds by the deposition of sand and shells, indeed, some of the compounds have been raised in this way with beneficial effects. There is one small swamp, namely, that behind Government House and the hospital, which urgently requires filling in, because in this neighbourhood a good number of Europeans are stationed, and it is from this swamp that they obtain the majority of the mosquitoes occurring in their houses.

To carry out the above suggested measures of dealing with mosquito breeding-places in Bathurst, it will be necessary in the first instance to appoint a small permanent sanitary staff for the purpose, whose sole work would be to destroy existing breeding-places and to prevent their recurrence. The sanitary staff should be under the control of the sanitary board, and its movements directed by the colonial surgeon ; it should at least consist of one inspector having a good knowledge of the mosquito, its larva, and its breeding-places ; under him a small gang of workmen, who could easily be taught to distinguish mosquito larva, and a cart for the removal of rubbish, tins, etc., from the various compounds would be required. The men, besides this work, would be employed in filling up holes and depressions, brushing out drains, and applying culicicides when required. It is especially necessary that such an inspector should understand when and where culicicides ought to be employed. Before any systematic work is undertaken, a preliminary removal of rubbish from houses and compounds and factory yards is essential. The rubbish so collected, which consists of old tins, bottles, iron pots, etc., will be of some value, as it can be utilized to fill up hollows and pools occurring throughout the town. After this preliminary removal, the work of the sanitary inspector will be :—

1. A systematic weekly inspection of all houses and compounds, for the purpose of searching out and dealing with breeding-places and preventing the accumulation of old tins, etc.
2. Systematic inspection of the street drains and boats on the foreshore.
3. Similar systematic inspection for natural breeding-places round the margins of the swamps.

Many of the breeding-places found in this way can be immediately destroyed by the inspector by one or more of the methods described above, others more difficult to deal with would have to be reported to the colonial surgeon.

The work of the anti-mosquito staff will naturally vary according to the season of the year. The inspection of yards and compounds must necessarily go on week by week all the year round, as mosquitoes are breeding in these places at all seasons of the year. A great deal of information will have to be obtained by the preliminary inspection as to which compounds are the most productive of mosquitoes,

Preliminary filling in of a grass-clogged ditch at Bathurst with old tins, scrap iron, etc., collected from compounds in the town, the source of many mosquitoes, chiefly of the *Anopheles* genus.

and such will require special attention. The public should be encouraged to report to the inspector when the mosquitoes are troublesome in order to help on the work.

The town drains will require special attention at the beginning, and more so at the end of the wet season (October and November), and it will be probably necessary to increase the staff of workmen at these times to keep them free from larvae, especially the grass-clogged drains. In the dry season (for seven months) they need scarcely any attention, with one most important exception, viz., the main drains to the sluice gates ; in these it has been shown that sea water collects at this time of the year, and in it many mosquito larvae are found ; regular application of kerosine oil to these drains, I believe, would be the most feasible method of treatment.

In the dry season, also, no special inspection of the swamps is necessary, as they are dried up at this time, but holes and depressions which have been found to act as breeding-places in the wet season can now be filled in with sand, etc., by the workmen. The swamps will require attention like the drains, more especially at the beginning and end of the wet season, when pools are shut off from the main mass of water ; I believe it will be found that in the height of the wet season few mosquitoes breed in these swamps. At this time the swamp is practically an open sheet of water exposed to winds, and is well-stocked with fish ; it will thus probably be more necessary to pay attention to the hollows in the streets and compounds bordering these swamps.

It cannot be expected that Bathurst will be appreciably freed from mosquitoes at once, solely by the energies of the mosquito brigade, because, it must be remembered, breeding-places occur which require more special attention than others ; for example, it will probably be necessary to deal with the small private wells and boats on the foreshore by legislation : also engineering work, such as levelling streets, making proper street drains, and filling in the swamps will necessarily take some time. **Still, Bathurst, by means of the special anti-mosquito staff, can certainly be made as free from mosquitoes all the year round as it is in the height of the dry season ; and, further, there is every reason to believe that mosquitoes can be diminished in numbers to a great extent, more especially by means of the house-to-house inspection.**

VI. THE COMMENCEMENT OF THE CAMPAIGN AGAINST MOSQUITOES IN BATHURST

In the early part of November, a preliminary report upon the sources of mosquitoes in Bathurst was submitted to His Excellency the Acting Governor, H. M. Brandford-Griffith, Esq. In this report the mosquito breeding-places of Bathurst were described, and methods applicable for their extermination were discussed; it was pointed out that the inauguration of a crusade against mosquitoes in Bathurst offered every hope of success, and at the same time, it was urged that any measures adopted in this direction should be of a permanent nature, and considered part of the sanitary work of the town.

On November 5, His Excellency very kindly gave the colonial surgeon, Dr. R. M. Forde, and myself an opportunity of discussing with him the various matters referred to in the report. At this meeting I was informed of the desire on the part of the Colonial Government to enter upon a crusade against the mosquito, and to undertake the necessary work. Soon after this date the plan of campaign was arranged; Mr. Thomas, the sanitary inspector, was selected for the part of 'anti-mosquito' inspector, and received special instruction in the work; an assistant inspector was appointed to take his place for the ordinary sanitary duties. A gang of ten of the sanitary board labourers, including one head-man, was appointed for special duty, and one of the sanitary carts was set aside for the work.

It was decided, in the first instance, to remove all the old tins, pots, and other rubbish from all yards and compounds; to this end notice was posted up throughout the town, informing the people of the nature of the work and inviting their co-operation and help.

On November 18, the preliminary removal of rubbish from the various compounds began. The work was started in Wellington Street, where most of the large trading stores are situated. At the outset the progress was slow, as the process of ridding these large business premises of discarded tin boxes and bottles took some time, in fact, at some of the factories it required two days to accomplish. After Wellington Street had been depleted of rubbish the work progressed more rapidly, from many of the compounds the natives had collected their old tins and pots and placed them in readiness for the sanitary carts, though, as was to be expected, it was found that many such-like articles were left behind in odd corners.

The rubbish thus collected from the compounds was utilized to fill in a deep

trench running behind the hospital, which has been already mentioned as being an excellent breeding-place for the mosquito; the old tins, which were well battered down, were then covered with sand from the shore.

After the removal of the rubbish from the larger compounds had been completed, a small gang of the labourers were employed in filling in two large pools near the cemetery, which were found breeding mosquitoes in great quantities; the third pool was left for a while until it could be replaced by a well, as this pool was required for the purpose of watering cattle. Besides this work a few foul wells were filled in. *Up to my departure from the Gambia, January* 10, *three hundred and sixty-three houses and compounds had been inspected, and from them one hundred and thirty-one cart loads of old tins, pots, and other rubbish were removed, and about two hundred and thirty yards of the trench behind the hospital had been filled in.*

On the return of the governor, Sir GEORGE DENTON, K.C.M.G., about the middle of December, other matters in connexion with the work were considered. It was decided that the work which had been started should be a permanent sanitary measure—*i.e.*, an inspector and sufficient labourers should be employed solely for the purpose of dealing with the destruction of mosquitoes. For this end the grant for sanitary work was increased to the extent of £200 per annum, and this annual amount was to be devoted to the work.

An ordinance was drawn up and passed by the legislative council in the early part of January, 1902. In this ordinance, which is to amend the Public Health Ordinance, 1887, powers were sought to enable the Governor in Council to make rules and regulations for various sanitary purposes. Some of the sections are of the utmost importance for the carrying on of the campaign against mosquitoes in Bathurst, because by them certain artificial breeding-places can be dealt with in a more thorough manner.

The sections relative to the anti-mosquito work include the following:—

11. *Breeding-Places for Mosquitoes*

Making provision for the removal, filling, or covering up of all drains, ditches, pools, swamps, holes, pits, depressions, cisterns, wells, tanks, barrels, tins, bottles, or broken pieces of bottles, and generally all receptacles, things, or places whatsoever, whether of a like nature to those before mentioned or otherwise, which are, or may be, capable of becoming breeding-places for mosquitoes or other noxious insects; and for the prevention (by the imposition of suitable penalties on the occupiers or owners of the premises on which the same are found, or other persons responsible) of the occurrence, accumulation, or continuance thereof.

12. *Wells*

Prohibiting or rendering, subject to conditions, the digging of wells in private compounds, and making provision for the cleansing, repairing, building up or re-building on proper principles of wells presently existing; also for the covering and keeping covered with wire-gauze lids, close pumps or other contrivances, as may be prescribed, of all wells whatsoever.

13. *Removal of Sand, etc.*

Preventing the removal or carrying away of any sand, shingle, rock, gravel, soil, or artificial protection from any part of the foreshore, or from any beach, bank, or public place whatsoever, without permission from some proper authority in such regulations to be indicated.

15. *Removal of Hulks*

Removing from any shores or beaches and open spaces, when thought advisable, all hulks, cutters, boats, canoes, timber, casks, rubbish, or any obstruction or objectionable article or thing whatsoever; or requiring the removal thereof by any persons judged to be responsible therefor.

17. *Inspection of Premises*

Providing for the inspection from time to time, and as often as may be thought expedient, of all lots, compounds, dwelling-houses, sheds, buildings, and premises whatsoever by such officers or persons as may be prescribed; subject to such conditions, safeguards, and requirements, whether as to obtaining an order under the hand of some Justice, the Chief Magistrate or other authority, or otherwise, as may be prescribed.

Dr. Forde informed me, in a letter dated February 17, that the removal of old pots and pans, etc., throughout the town had been completed, and that the inspector had now begun his regular weekly inspection of yards and compounds; also a gang of men had started filling in some of the grass-clogged drains, and the filling in of all large pools near the cemetery had now been accomplished.

I understand also that special sanitary regulations have been drawn up, but as yet I have not seen a copy.

REGULATIONS UNDER SECTION 2 OF THE PUBLIC HEALTH AMENDMENT ORDINANCE, 1902 (No. 1 of 1902)

I. (1) The occupier, and if no occupier, then the owner, of any lot, yard, compound, or other parcel of land whatsoever, shall fill up, or cause to be filled up, all drains, ditches, pools, holes, pits, irregularities, and depressions in the ground or surface of such lot, yard, compound, or parcel of land which may be of such a nature as to cause the accumulation or stagnation of water.

(2) No person shall, unless for building or other necessary or reasonable purposes, dig or make any drain, hole, irregularity, or depression in any portion of ground whatsover so as to cause or allow the accumulation or stagnation of water; all building and other such operations shall be conducted in such a manner as, so far as possible, to avoid causing such holes, irregularities, or depressions as aforesaid.

(3) Sand for making mortar or other building purposes shall in no case be taken out of the ground or soil of any lot or compound, but may be taken from the beach below or near low water mark.

II. (1) No person shall place on any wall or building, any bottles, or broken pieces of bottle, or other articles unless broken into small fragments so as to be incapable of containing water.

(2) The occupier, or if no occupier, then the owner of any building, or of any lot, yard, compound, or parcel of land whatsoever, shall cause all bottles, broken pieces of bottle, and other articles on or attached to such wall or building to be removed or broken into small fragments so as to be incapable of containing water.

III. The occupier, or if no occupier, then the owner of any lot, yard, compound, or parcel of land shall, on being required so to do by a notice under the hand of the Chairman of the Board of Health, cause all discarded tins, pots, bottles, calabashes, and all other discarded articles capable of containing water in or on such lot or parcel of land to be collected in readiness for removal by the carts of the Board of Health.

IV. The owner of any premises wherein there is now, or may hereafter be dug, any well, shall within thirty days after service upon him of a notice under the hand of the Chairman of the Board of Health requiring him so to do, cover such well with a wooden, wire-gauze, or other cover to the satisfaction of the Inspector of Nuisances.

V. The occupier, and if no occupier, then the owner, of any house, shed, or other building whatsoever, or of any lot, yard, compound, garden, or other parcel of land whatsoever, shall cause every cistern, tank, barrel, or other receptacle whatsoever holding or capable of holding water on such premises to be maintained in such a manner as not to be or to be capable of becoming a breeding-place for mosquitoes or other noxious insects.

VI. All hulks or boats hauled up on any beach or open space within the town of Bathurst, shall, so far as possible, be turned keel upwards; boats in course of construction or undergoing repair shall be exempt from this requirement, provided always that such construction or repairs are carried on with reasonable expedition, and the boats are not allowed to become breeding-places for mosquitoes by the accumulation of water therein.

In cases where it shall be found impracticable to turn any lighter or boat keel upwards, it shall be the duty of the owner thereof to take due and sufficient measures to the satisfaction of the Chairman of the Board of Health to prevent the accumulation of water therein.

'Boats' in this regulation shall include canoes, cutters, and any other small craft whatsoever.

VII. The owner of any bakehouse shall once in every six months cause the complete inside of such bakehouse to be whitewashed.

VIII. Section 49 of the Public Health Ordinance, 1887, shall be construed and is hereby declared to apply to the entry of premises for any purpose whatsoever connected with the due sanitation thereof and the enforcement of any requirement or prohibition in the said Ordinance or hereinafter in these Rules contained.

Any costs or expenses incurred in obtaining an order under the hand of the Chief Magistrate or of a Justice of the Peace as in the said Section provided, shall be recoverable against the occupier or owner of the premises in like manner as other costs and expenses are recoverable under Section 112 of the said Ordinance.

IX. If on the complaint of one or more residents it shall appear and be ascertained to the satisfaction of the Chief Magistrate or two Justices of the Peace that any person has been annoying such complainant or complainants or others by unreasonably, and at late hours of the night, playing upon any string, brass, or other musical instruments, or by singing, the Chief Magistrate or the two Justices aforesaid may in their discretion dismiss such offender with a warning, or if the offence appear to him or them to have been of repeated occurrence, whether before or after such warning, or attended by any aggravating circumstances whatsoever, fine such offender in such sum as they shall consider suitable, not exceeding ten shillings.

X. Canes shall not be dragged along Russell Street or Wellington Street.

XI. Any person contravening or wilfully or negligently failing to comply with any of the provisions contained in these regulations after service on him of a notice to that effect, under the hand of the Chairman of the Board of Health, shall be liable on conviction before the Chief Magistrate or two Justices of the Peace to a penalty which (save where it may be otherwise provided in the case of any particular offence) shall extend to, but not exceed, the sum of five pounds for any one offence, or in default of payment thereof to imprisonment with or without hard labour for any period not exceeding one month.

Passed in the Executive Council this Seventh day of July, 1902.

GEORGE C. DENTON, *Governor*

APPENDIX

REPORT ON A COLLECTION OF MOSQUITOES OR CULICIDAE, ETC., FROM GAMBIA, AND DESCRIPTIONS OF NEW SPECIES

By F. V. THEOBALD, M.A.

THE collection of Culicidae and other blood-sucking Diptera, made by Dr. DUTTON during his visit to Gambia, contains some three hundred Culicidae, included in the following genera : *Anopheles*,* *Stegomyia*, *Culex*, *Mansonia*, *Uranotaenia*, and *Corethra*. Altogether there are seventeen species of Culicidae as follows : three *Anopheles*, three *Stegomyia*, seven *Culex*, one *Mansonia*, and a single *Uranotaenia* and *Corethra*. There is also a distinct variety of *Anopheles costalis* and *Anopheles funestus*. Besides Culicidae, there are some specimens of Psychodidae, or Owl Midges, of the genus *Phlebotomus*, probably a new species, and several specimens of the common West African gadfly (*Tabanus dorsovitta* WALKER). A number of one of the Tsetse flies, *Glossina longipalpis* WIEDMANN var., *tachinoides*, WESTWOOD, were also taken.

This insect, closely related to the Tsetse fly (*Glossina morsitans*), is called by Dr. DUTTON the small Mangrove fly. It is very prevalent up the Gambia river, and comes on board the launches and bites viciously. It is of particular interest, as the case of Trypanosoma Dr. DUTTON found in Bathurst was in an Englishman, who was master of the Government launch, living on board, and was frequently bitten by this species of *Glossina*. It is quite possible that this species of *Glossina* acts in the same way as *G. morsitans* in the animal Tsetse disease.

The collection contains no new *Anopheles* but three distinct varities, three new species of *Culex*, and a distinct variety of a previously known one, also a new *Stegomyia* and a *Corethra*. The series of *Anopheles funestus* is most interesting, as it shows very great variation, particularly in the colour of the vein-scales and the position of the cross-veins, which I had found constant before in this species, and which I took to be of some specific value. Great variation is also to be noticed in a large series of *Culex Duttoni* (THEO.) This species is of particular interest, as it serves as one of the intermediate hosts of *Filaria nocturna*. *Culex fatigans* (WIED.) was also found to act as an intermediate host of this *Haematozoon*. In a new banded proboscis species (*Culex anarmostus*) a filaria (sp. incert.) was found in the thoracic mucles. A list of the species, with notes and the descriptions of the *Stegomyia*, *Culex*, and *Corethra*, are here appended, and also a description of the varieties of previously known species.

LIST OF CULICIDAE AND OTHER DIPTERA TAKEN AND BRED BY DR. DUTTON

A. CULICIDAE.

1. *Anopheles costalis.* LOEW.
1A. *Anopheles costalis.* Var. *melas* n.v.
2. *Anopheles pharoensis.* THEOBALD.
3. *Anopheles funestus.* GILES.
3A. *Anopheles funestus.* Var. *umbrosus* n.v.
3A. *Anopheles funestus.* Var. *subumbrosus* n.v.
4. *Stegomyia fasciata.* FABRICIUS.
5. *Stegomyia sugens.* WIEDMANN.

* The old genus *Anopheles* is now subdivided into several genera ; *costalis* comes in Pyretophorus, *pharoensis* in Cellia, and *funestus* in Myzomyia.

h

6. *Stegomyia albocephala.* N. sp.
7. *Culex hirsutipalpis.* THEOBALD.
8. *Culex annulioris.* THEOBALD. Var. *gambiensis.* n.v.
9. *Culex duttoni.* THEOBALD.
10. *Culex anarmostus.* N. sp.
11. *Culex thalassius.* N. sp.
12. *Culex tigripes.* GRANDPRE.
13. *Culex fatigans.* WIEDEMAN.
14. *Culex euclastus.* N. sp.
15. *Lasioconops poicilipes.* N. sp.
16. *Mansonia uniformis.* THEOBALD.
17. *Uranotaenia albocephala.* THEOBALD.
18. *Corethra ceratopogones.* N. sp.

B. PSYCHODIDAE.

Phlebotomus sp. ?

C. TABANIDAE

Tabanus dorsovitta. WALKER.

D. GLOSSINIDAE

Glossina longipalpis. (WIED.)—var. *tachinoides.* WESTWOOD.

CULICIDAE

I. *Anopheles costalis.* LOEW
A. gambiensis. GILES

(Ent. Zeit. Berlin, 55 (1866) LOEW; Mono. Culicid. I. 157 (1901) THEO. Hand Bk. Gnats, 2nd Edn. GILES, 1902. (=*A. gambiensis.*)

A number of this species from Bathurst, many of them caught in the barracks, prison, and police quarters, Government House; some bred from larvae obtained from a large pool sixteen to eighteen feet in diameter.

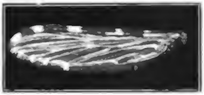
The specimens show some variation in regard to the intensity of the costal spots and leg ornamentation. One very marked melanic variety occurs, which is described below. The specimens were taken in October, November, and December. None were found at Baia or McCarthy Island. At Bathurst, Dr. Dutton only obtained *A. costalis* and a few *A. pharoensis.* THEO. This species also occurs at Cape St. Mary, seven miles from Bathurst, where there are a few artificial breeding-places. Colonel GILES has described as a distinct species a specimen of *A. costalis* sent me from Gambia by Dr. BUDGETT.

IA. *Anopheles costalis.* LOEW
Variety *melas*

Thorax dark brown to almost black, with narrow-curved pale golden scales as in the type; palpi, with *four* pale bands, *very* narrow; the fourth on the apex of the palpi, very scaly at their base; the two apical bands are close together, but quite distinct. Abdomen deep black, with pale hairs, golden at the apex. Legs prominently black, spotted and banded; forelegs with a trace of pale spots on the femora as in the type, pale spots on tibiae, and a narrow band-like spot on the metatarsi, a yellow band involving both sides of the joints at the metatarsus and first tarsal, and at the first tarsal and second tarsal; in the mid-legs the tibiae are spotted, but the tarsal banding is not distinct, nor are the tarsi banded in the hind legs, and the tibiae and femora spots are not so well defined.

WINGS OF GAMBIAN *CULICIDAE*

1



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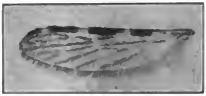

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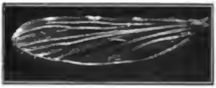

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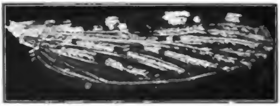

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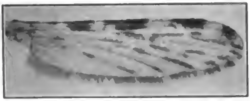

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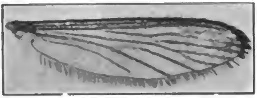

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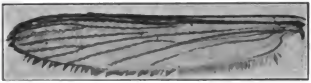

10

1. *Anopheles funestus.* Giles. ♀ var.
2. *Anopheles funestus.* ♀ Typical.
3. *Anopheles funestus.* ♂
4. *Anopheles funestus.* Var. *anisochloros.*
5. *Anopheles rhodesiensis.* Theo. ♀
6. *Anopheles pharoensis.* Theo. ♀
7. *Anopheles costalis.* Loew. ♀
8. *Culex thalassius.* N. sp.
9. *Culex euclaston.* N. sp.
10. *Anopheles rhodesiensis.* Theo. ♂

(All × nine times except No. 10 which is × twelve).

a

b

Fig. 1

Anopheles funestus. Giles
a. Head of ♂ ; *b.* Head of ♀

Fig. 2

Wing of ♀ *A. costalis* to show venation, not markings.
× 18

Wings with black- and yellow-scaled areas, the former predominating; the costa is deep black, the second spot only appearing on the costa as a small, almost white, spot; there are also two small pale spots on the costa towards the base of the wing; on the first long vein are six pale spots, one under the white costal spot, the other arranged much as in the type, but the black areas are pronounced; the greater part of the third long vein is pale and the second mostly dark scaled, except for the pale patch at the base of the fork and a pale patch towards the apex of its lower branch; most of the fourth dark scaled, the lower branch of the fork having two pale patches. The fifth mostly pale scaled, but with three dark patches on the upper branch and a small one at the apex and another at the base of the lower and its stem; sixth with three black spots; fringe spots very indistinct. Fork-cells and cross-veins as in the type.

Length—5 mm. *Habitat*—Gambia (Dutton). *Time of capture*—December.

Observations—Described from a single female in perfect condition. It forms a very distinct melanic variety. The chief difference from a typical *A. costalis* is the absence of pale costal spots, one only reaching the actual costa, except at the base; the whole wing field is darker, and the legs with more pronounced spotting. The markings in the first long vein are, however, typical of the species.

II. *Anopheles pharoensis.* Theobald

(*Mono. Culicid.* I, 169 (1901). Theobald)

Nine specimens (seven ♀'s and two ♂'s) of this beautiful *Anopheles* from the following localities: the barracks and prison, Bathurst, and on a marsh at the back of the town, McCarthy Island. A specimen was also hatched from a larva taken in a pool, fifteen yards across, at Box Bar. The specimens show considerable variation in size, one only measuring 5 mm.; there is also marked variation in colour, due evidently to some containing blood. One large pool alone, some way from the town of Bathurst, acted as a breeding ground of this species. This large *Anopheles* also occurrs at Cairo, Central Africa, and in Palestine, and probably occurs all over Africa and in other parts of Asia.

III. *Anopheles funestus.* Giles

(*Mem. II, Liv. School of Trop. Med.* p. 50 (1900), Giles; *Mono. Culicid.* I, p. 182 (1901), Theobald)

A large series of ♂'s and ♀'s of this species were taken in the following places:—Baia, the Cape, and McCarthy Island. The species occurs in native huts, and many were taken on the walls in the prison and in Government House at McCarthy Island. They were mostly taken in December. Both at Baia and McCarthy Island there were no ordinary or artificial breeding-grounds about, except here and there a large marsh. At Baia the marsh was about two miles away from the town. At Cape St. Mary, seven miles from Bathurst, this small *Anopheles* occurs in numbers, and the larvae are here found in rice swamps. This species, to some extent, resembles *A. rhodesiensis*, but can at once be told from it by the pale fringe spots and by the pale scaled areas to the wings, and the more pronounced dark patches. The white palpal bands are also, it seems, wider apart in *A. rhodesiensis*. Several of the specimens of *A. funestus* in this collection present well-marked deviations from the type. Speaking generally, the pale and dark scaled areas on the veins are not so pronounced, and the base of the fork-cells have not quite the same relative positions. In my monograph (Vol. I, p. 186) I pointed out that one of the characters separating *Funestus* from the larger *Rhodesiensis*, was the position of the cross-veins, this does not hold good, for in the *Funestus* from Gambia I find the cross-veins in some like *Funestus* as I described, in others like *Rhodesiensis*. The supernumerary and mid may be either in one line as the mid may be in advance of the supernumerary and posterior. *Rhodesiensis* has, however, *all* the vein scales dark, and the fringe unspotted, and the third long vein always dark. The wings are *always* black at the base of the costa, whereas, in most *Funestus* there is a pale costal spot near the base. *Funestus* is also smaller than *Rhodesiensis*; the latter has so far only been sent from Mashonaland.

IIIa. *A. funestus.* Giles
Var. *Umbrosus.* Theo.

Costa black at the base, unbroken by the typical small pale spot. Veins with the dusky scales predominating; the pale scaled areas restricted to the region of the cross-veins and base of the fork-cells and on the fifth long vein; *the third long vein dark as in Rhodesiensis.* Wing fringe spotted as in the type, but not so prominently.

IIIb. *A. funestus.* Giles
Var. *Subumbrosus.* Theo.

Costa black at the base, unbroken by any pale spot. Dusky scales predominating, but not contrasted as in the type with the pale scaled areas. *Third long vein pale-scaled in the middle,* and pale scaled areas also on the fourth, fifth, and sixth.

IV. *Stegomyia fasciata*
(*Syst. Antl.* 36, 13, 1895. *Mono. Culic.* I, 289, 1901. Theo.)

This species is evidently common in Gambia, specimens showing great variation in size were taken in Bathurst in numbers: some in native huts, and also in European dwellings. Many seem to have been hatched out from larvae taken in a tub of well water, and others from a canoe. This species was taken in October, November, and December, and was observed feeding, as recorded elsewhere,* during the daytime (4 p.m.)

V. *Stegomyia sugens.* (Wied.)
Auss. Zweiflug Insec. 545 (1828) Wied; *Mono. Culic.* 300 (1901) Theo.

Three ♂'s and two ♀'s taken near Bathurst. This *Stegomyia* can easily be told by the spots on the mesonotum and the pale band on the femora near the apex. Hatched out from larvae taken in ground-nut gutters during November.

VI. *Stegomyia albocephala.* N. sp.

Head covered with flat dull white scales, a small dusky patch on each side and a posterior semi-circular area of dark upright forked scales.

Thorax deep rich brown covered with scattered golden scales, showing more or less two dark eyes like spots; scutellum with small flat white scales. Abdomen black with narrow basal white bands. Legs black, the hind tibiae with a marked apical white band.

♀. Head brown, covered with dull white flat scales, with a silvery sheen, a small patch of black scales on the border about the middle of the eyes, and dull black scales at the sides, posteriorly are black upright forked scales, giving the head a dark appearance of semicircular form, in front the upright forked scales are yellow. Proboscis, palpi, and antennae deep blackish-brown; palpi with a trace of a pale band on its basal half, two apical joints nearly equal, with black hair-tufts and also black hairs at the apex of the anti-penultimate joint.

Thorax rich deep-brown with scattered golden narrow-curved scales, and showing in certain lights two dark eye-like patches on the ground surface; scutellum covered with small flat shiny creamy-white scales; pleurae brown with patches of grey scales; metanotum deep clear brown.

Abdomen black with basal white bands which spread out laterally; venter black with broad basal white bands; densely clothed with long brown hairs.

Legs black, unbanded, except for a clear, rather broad, white apical band to the hind tibiae. Coxae brown, bases and venter of the femora grey, ungues of the fore and mid legs unequal, the larger uniserrated, the smaller (?) hind ungues rather long, curved, equal, and simple. Wings with the first submarginal

* *Mono. Culicidae,* Vol. i, p. 60. Theobald. (1901).

cell longer and narrower than the second posterior cell, its base nearer the base of the wing than that of the latter, its stem not quite so long as the cell ; stem of the second posterior as long as the cell ; posterior cross-vein about half its own length distant from the mid cross-vein ; halteres with yellow stem and slightly fuscous knob.

Length—4·5 mm. *Habitat.*—Gambia. *Time of appearance*—November.

Observations—Described from a single perfect male, bred by Dr. Dutton from a larva found in a canoe. This *Stegomyia* very closely resembles *Culex univittatus* mihi, and might readily be mistaken for it, on account of the conspicuous hind tibial banding, but an examination of the head and scutellum reveals flat scales only. The pale head and white shiny scutellum with the brown thorax form also striking characters.

VII. *Culex hirsutipalpis.* Theobald
(*Mono. Culicid.*, Vol. i, p. 378 (1901). Theobald)

A series of this species, which I described from some specimens from Mashonaland, were hatched out by Dr. Dutton from larvae taken in the water of ground-nut insect traps, *i.e.*, gutters full of water around the ground-nuts. Other specimens were hatched from a small dug-out pool in a rice swamp. The specimens hatched out in November and December.

The abdomen in the ♂ is much better marked than in the original type. A fresh description of the male is therefore appended.

♂. Palpi black, with four white bands, the two apical ones on the base of the last two joints narrow ; last two joints with dense tufts of hair, hairs black, except at the apex, where they are pallid ; the antipenultimate joint is also hairy down to near the first white band ; antennae banded black and grey, with deep-brown plume-hairs ; proboscis with a narrow white band.

Thorax as in ♀. Abdomen black, the second to the fifth segments with basal white bands, the sixth and seventh have the basal band spreading down each side, the last segment with a basal median white spot ; apical hairs golden ; there are also white lateral linear prolongations of the basal bands to each segment ; venter covered with pale creamy yellow scales ; legs much as in the ♀ ; fore and mid ungues unequal, both uniserrated.

Wings paler than in the ♀ ; first submarginal cell longer and narrower than the second posterior cell, the bases of the fork-cells nearly level ; stem of the first submarginal rather more than half the length of the cell ; stem of the second posterior as long as the cell ; posterior cross-vein about its own length distant from the mid cross-vein. Halteres pale, but the knob slightly tinged.

Note.—Fresh specimens are much darker than old ones. The proboscis band is narrower in the male than in the female.

VIII. *Culex annulioris.* Theobald
Var. *Gambiensis.* *n.v.*
(*Mono. Culicid.*, Vol. i, p. 371)

Proboscis with white band. Thorax brown with narrow-curved pale brown and grey scales on the front two-thirds ; narrow-curved black ones on the hinder third of the mesonotum ; the pale brown scales in front form more or less a distinct median line, with a narrow pale scaled line on each side and an indistinct darker broad line on each side of the narrow pale line, bounded laterally by mostly pale scales ; the scutellum, as in the type, with small black scales at the base of the mid-lobe and grey ones on the apical portion ; metanotum bright amber brown. The abdomen is like the type, but the triangular basal white spots are very indistinct, but can be detected on each segment by a few white scales when examined under the microscope.

The band on the proboscis is not quite so broad as in the type, and the stem of the first submarginal cell is very nearly half the length of the cell.

Length—5·5 mm. *Habitat*—Gambia (DUTTON). *Time of capture*—January.

Observations—Described from a single female hatched from a larva taken in water in a rice field.

It resembles the type except in regard to the colour of the thoracic scales, the thorax is characteristically ornamented, under a lens the first part (two-thirds) looks ashy grey, but more or less ornamentation, as described, may be seen on careful examination, the paler anterior area is clearly marked off from the dark scaled posterior third. It is undoubtedly only a variety of the species I described as *C. annulioris*, from Salisbury, Mashonaland.

In the structural figure of this species in the *Monograph of Culicidae*, fig. 127, p. 372, vol. I, I figured the palpi as three-jointed, the apical joint being characteristically swollen and truncated, this is really the penultimate joint, the apical joint was missing, I find the apical joint is long and thick.

IX. *Culex duttoni.* THEOBALD

Mono. Culicid. II, p. 318 (1901). THEO.

A large series of this mosquito were taken at McCarthy Island and Bathurst Some were hatched out from larvae taken in a canoe on the foreshore, others from a tub of well water during October, November, December, and January. This is evidently a common West African insect along the coast; I have not at present seen any from inland. It was found to be one of the hosts of *Filaria nocturna* by Dr. DUTTON.

This species is subject to considerable variation, both in size and in thoracic ornamentation. In some specimens brought back by Dr. DUTTON the thorax shows no ornamentation at all, others have the thorax adorned as I described in the *Monograph of the Culicidae*.

X. *Culex anarmostus.* N. sp.

Thorax dark brown to brown, with two darker median parallel lines on the denuded surface, covered with pale, dull golden, narrow-curved scales, showing faint longitudinal arrangement. Proboscis with a pale creamy band. Abdomen brown, with curved basal white bands. Legs brown, with faint apical and basal pale banding. Ungues equal and simple.

♀. Head brown, with narrow-curved, pale, creamy-grey scales, brown upright forked ones and small flat white ones at the sides, and whitish curved ones round the eyes. Proboscis brown, with a median pale band very distinct beneath; palpi black, with a few white scales; clypeus black; antennae dark brown, basal joint testaceous. Thorax brown to almost black, covered with narrow golden curved scales somewhat paler behind, to some extent arranged longitudinally; scutellum pale brown, with pale narrow-curved scales; metanotum deep brown; pleurae pale brown and cinerous, with a few patches of grey scales.

Abdomen deep brown, with curved white to creamy basal bands; first segment nude, save for two median patches of black scales; border-bristles pale; venter white, with narrow apical border of brown scales.

Legs brown; femora pale ventrally, apex of tibiae white, base and apex of metatarsi and first two tarsals pale banded, also a white knee spot on the hind legs; femora and tibiae bristly; ungues equal and simple; hind tibiae about the same length as the hind metatarsi. Wings with brown scales, those on the third and fifth being the darkest; first submarginal cell longer and a little narrower than the second posterior cell, its base a little nearer the base of the wing than that of the latter, its stem half the length of the cell; stem of the second posterior about two-third the length of the cell; posterior cross-vein about its own length distant from the mid cross-vein. *The medium vein scales of the third, fifth,* and to some extent the lower branch of the second fork-cell, rather larger than in most *Culex*, and very dark. Halteres pale.

Length—4·5 mm.

Habitat—Freetown, Sierra Leone (AUSTEN), Gambia (DUTTON).

Time of capture—September (Freetown), AUSTEN ; Gambia (in November), DUTTON.

Observations—Described from a single female from Freetown ; bred from water in a drain by Mr. AUSTEN.

A specimen sent me by Dr. DUTTON, from Gambia, is evidently this species, but it is rather too damaged to say definitely. Dr. DUTTON found a filarial embryo in the thoracic muscles.

XI. *Culex thalassius.* N. sp.

Proboscis with a narrow median white band. Thorax dark-brown, with narrow deep golden-brown curved scales. Abdomen dark brownish-black, with narrow basal grey bands, often absent ; penultimate segment with lateral white spots only ; pleurae very pale grey. Legs deep brown, with faint pale bands to some of the mid and fore tarsi ; apices of tibiae pale, hind legs unbanded. Bases of the fork-cells nearly level.

♀. Head deep brown, with narrow-curved, pale greyish scales and black upright forked ones ; palpi black ; proboscis black, with a narrow distinct pale band ; antennae brown ; clypeus black.

Thorax deep brown, with narrow rich brown curved scales ; scutellum brown, with narrow golden-brown curved scales, and deep brown border-bristles ; pleurae very pale and shiny grey ; metanotum deep brown. Abdomen black, with narrow basal white bands, or unbanded with traces of basal white lateral spots, venter dark, with broad basal grey bands.

Legs black, bases pallid, also venter of femora, apex of femora, and to some extent the tibia, pale ; tarsi and metatarsi with *narrow* pale basal bands, indistinct on the last two tarsi ; hind metatarsi and tibiae of about equal length.

Wings with the veins with brown scales ; fork-cells rather short, their bases about level ; the first submarginal a little longer and narrower than the second posterior, its stem a little more than half the length of the cell ; stem of the second posterior about two-thirds the length of the cell ; posterior cross-vein nearly twice its own length distant from the mid.

Length—4·5 mm. *Habitat*—Gambia. *Time of capture*—October and November.

Observations—Described from a series taken and bred by Dr. DUTTON. The larvae were mostly taken in a drain of tidal water, and others from a pool in a mangrove swamp ; others from a canoe on the foreshore, and some from a pool of tidal water that had soaked through sand into a drain.

The species is very variable ; some show distinct abdominal banding, others none at all. It somewhat resembles *C. duttoni*, but is smaller, more fragile, and the legs have only faint basal banding, and the fork-cells are slightly different.

This species and *C. duttoni* come very close together, but they are certainly distinct.

XII. *Culex tigripes.* GRANDPRE

(*Les Moustiques.* (1901.) GRANDPRE. *Mono. Culicid.* II, p. 34. (1901.) THEOBALD)

A series of ten ♂'s and ♀'s taken at Bathurst and McCarthy Island during October. Some specimens were taken on the sides of a discarded well ; the majority were hatched from larvae taken in canoes, and also from a pool.

This large spotted-legged *Culex*, with its apical pale abdominal bands, seems to be generally distributed over Africa, but so far has not been recorded further south than Natal, as well as occurring in Mauritius and Australia. It is the species that Dr. BANCROFT calls the 'long-lived mosquito.' Some of the specimens are very small, not more than 5·5 to 6 mm., others are as much as 7 mm.

XIII. *Culex fatigans.* Wied.

This common household *Culex* occurs in abundance in Bathurst, and was taken in numbers as usual indoors. Some were hatched from larvae 'from an old tin,' others 'from a well,' 'from a rain tub,' 'from water in rice field at Cape St. Mary,' 'from well in Government House with heaps of green slime.' This species seems abundant in the prison at Bathurst, and has been shown by Dr. Dutton to be the intermediate host of *Filaria nocturna*, as well as *Culex duttoni*.

They were taken in October, November, December, and January.

XIV. *Culex euclastus.* N. sp.

Head brown with grey scales, most distinct around the eyes. Thorax brown with tawny-brown scales. Abdomen brown, unbanded, with basal white lateral spots, which show dorsally on the last few segments; legs brown, unbanded, basally grey. Sixth long vein rather close to the fifth.

♀. Head dark brown with narrow-curved dull-grey scales, rather wider and paler around the eyes, and with dark upright forked-scales; proboscis and palpi dark brown: antennae dark brown, basal joint paler. Thorax brown with very small narrow-curved scales of a fawny-brown to dull brownish-grey hue, and with dark-brown bristles; scutellum paler brown with narrow-curved grey scales; metanotum brown; pleurae pallid.

Abdomen brown, unbanded, with basal white lateral spots, which are pronounced, and which show dorsally on the last few segments. Venter, brown with dull grey basal bands; border-bristles and hairs brown, except at the apex, where they are pallid; the denuded surface of the abdomen has a shiny and somewhat pale steel colour.

Legs brown, unbanded, a faint pale knee spot on the hind legs and traces of a pale apical tibial spot; bases of the legs and centre of the femora pallid; hind metatarsi about the same length as the hind tibiae; legs with a few bristles. Ungues small, equal and simple. Wings with deep-brown scales, costa very dark; first submarginal cell longer and narrower than the second posterior cell, its base very slightly nearer the base of the wing than that of the second posterior cell; its stem rather less than half the length of the cell; stem of the second posterior rather more than two-thirds the length of the cell; posterior cross-vein longer than the mid, rather more than its own length distant from it; the sixth long vein runs parallel with the fifth at its base, and is rather closer than usual to it. Halteres brown, with dense white scales.

Length—4 mm. *Habitat*—Gambia. *Time of capture*—October.

Observations—Described from two perfect ♀'s bred by Dr. Dutton from larvae taken from pools at Box Bar.

It is a very small fragile-looking species, unlike any other I have seen from Africa, and to some extent approaches *Culex nigritulus* Zett, but is very distinct in regard to thoracic scale structure and venation. The type is deposited in the British Museum (Nat. Hist.) Collection.

Genus *Lasioconops.* New. gen.

Head clothed with similar scales to *Culex*; antennae with the basal joint with a few scales; palpi short in both sexes. Thorax clothed with narrow-curved scales. Abdomen clothed with flat scales and with *large projecting flat lateral scales, with deeply dentate apices*, in more or less tufts. Wings with typical *Culex* scales and venation.

This genus is separated from *Culex* on account of the peculiar and characteristic lateral scales on the abdomen, which give the insect a ragged appearance.

A single species only at present occurs, *L. poicilipes* from West Africa. The ♂ is unknown.

XV. *Lasioconops poicilipes.* N. sp.

Anterior half of thorax with ashy grey scales and chestnut brown ones, the former towards the edge of the pale area, posterior part of the thorax dark brown with brown scales. Abdomen black, with basal white bands. Proboscis brown, with a pale median band. Legs deep brown, the femora mottled with creamy scales, the tibiae with a row of pale spots, metatarsi and tarsi with narrow basal pale bands, which to some extent involve the apices of the preceding segments.

♀. Head dark brown, with narrow-curved pale grey scales, brown and ochraceous forked scales and small flat grey ones at the sides; antennae brown, basal joint black on the inside, with small white scales, and with a grey sheen on the outside, second joint bright testaceous; palpi black scaled, with apical grey scales; proboscis black scaled, with a pale median band; clypeus deep brown, with frosty sheen.

Thorax black, the anterior two-thirds clothed with narrow-curved grey scales, palest at the posterior edge of this pale scaled area, where they form a wavy line; posterior portion of the mesonotum with narrow-curved black and brown scales and numerous black bristles. Scutellum brown, with narrow curved dull creamy scales, and with eight black border-bristles to the mid lobe; pleurae black, with patches of white scales and pale creamy hairs.

Abdomen black, with narrow basal bands of white scales and very large and peculiar white and ochraceous lateral projecting scales; posterior border-bristles golden, short; venter black, with white scales. Legs dark brown, the femora spotted and mottled with pale scales, the tibiae with small creamy spots; metatarsi and tarsi dark brown, with narrow pale ochraceous bands involving both sides of the joints.

Wings with typical brown *Culex* scales; surface of the wing with minute bristles; first submarginal cell longer and narrower than the second posterior cell, its base nearer the base of the wing than that of the latter, its stem about one-fourth the length of the cell; stem of the second posterior not quite one-third the length of the cell.

Supernumerary cross-vein not level with the mid cross-vein, a little nearer the base of the wing; posterior cross-vein about two-and-a-half times its own length from the mid cross-vein; sixth vein rather densely scaled. Halteres dusky ochre.

Length—6 mm. *Habitat*—Bonny, West Africa (Annett), and Gambia (Dutton). *Time of capture*—July (Annett), December (Dutton).

Observations—Described from a single ♀, somewhat denuded but easily told from all other Culicidae by the curious abdominal lateral scales, which are certainly of generic importance. The spotted legs give it some resemblance to *Culex tigripes*, but the banded tarsi and proboscis and general ornamentation will at once separate it.

XVI. *Mansonia uniformis.* Theobald

Mansonia africanus. Theobald

(*Mono. Culicid.* II, p. 180 (*Uniformis*) and p. 187 (*Africanus*) (1901) Theo.)

The collection contains ten specimens of this abundant African *Mansonia*. They were taken at McCarthy Island, in the marsh at the back of the town, and were noticed to bite very viciously. A single specimen was also taken in the prison at Bathurst, in October, the others were taken in December. Dr. Daniels has shown this *Mansonia* to be an intermediate host of the *Filaria*.

After carefully comparing a fresh series of South Indian and Ceylon *Mansonia* with the ones I described as *M. africanus* (*Mono. Culicid.* II, p. 187), I am convinced they are the same as the Indian *M. uniformis*. *M. africanus* must, therefore, sink as a synonym of *M. uniformis*. The thoracic ornamentation very soon becomes destroyed, and the thorax has then a non-ornamented or uniform appearance.

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XVII. *Uranotaenia caeruleocephala.* THEOBALD
(*Mono. Culicid.* II., p. 256, 1901

I have described the ♀ of this species but not the ♂, a description of which is here given :—

♂. Thorax like the female, but the metallic patches in front and the lines in front of the wings very pale blue in certain lights. The head is brown and deep violet in the middle, with pale blue scales on each side; palpi brown, proboscis brown, swollen at the apex; antennae banded brown and deep brown, densely brown plumed. Abdomen showing a pale apical ventral spot on the fifth segment; paler ventrally than dorsally; fore ungues unequal, the larger sickle-shaped simple; mid and hind apparently equal and simple, irregularly curved. Wings with brown veins, a line of metallic flat pale blue and violet scales at the base of the costa and another at the base of the fifth long vein, posterior cross-vein twice its own length distant from the mid cross-vein; halteres with pale stem and brown knob.

Length—3 to 3·5 mm. *Habitat*—Gambia (BURDETT ♀) and (DUTTON ♂). *Time of capture*—December.

Observations—The ♂ is described from two fairly perfect specimens caught in a marsh behind the town on McCarthy Island. I feel certain from the thoracic ornamentation it is the male of *U. caeruleocephala* (mihi) described from Bonny. The chief difference from the female lies in the head being deep violet in the middle, instead of pale blue all over. The markedly bright brown thorax with the metallic white and pale blue ornamentation should at once separate it. I had to mount some of the legs of the ♂ type in balsam to make anything of the ungues. In doing so I misplaced them, so am not sure if the anterior or mid ungues are unequal.

XVIII. *Corethra ceratopogones.* N. sp.

♀. Thorax pale brown to fawn with darker brown markings; metanotum pale chestnut-brown; pleurae pale fawn and cinerous; head brown, proboscis and palpi brown, with numerous rather long brown hairs; antennae banded brown and grey. Abdomen very pale fawn to cinerous, with narrow dark brown apical borders to the segments, and dark brown at the sides, only partly, however, on the last two apical segments; abdomen hairy; apex dark brown; lamellae brown.

Legs multibanded, with brown and frosty grey on the femora and tibiae; fore femora with six dark bands and also the fore tibiae, apex and the basal band of both, pale; metatarsus and first three tarsi banded with dark brown in the middle, apical joint pale, ungues very small, simple, and equal; mid femora with eight dark bands, tibiae with six, the tarsal are broadest, base and apex of both joints pale; metatarsi and tarsi with very broad dark median bands; ungues small, equal, and simple; hind femora with eight, and hind tibiae with seven dark bands, base and apex of each pale, metatarsus with two median dark bands, tarsi with a single median dark band. Ungues small, equal, and simple. Wings densely clothed with long brown hair-like scales, with three dusky patches on the costa, the median one where the sub-costal joins the costa spreading on to it, the apical one spreading on to the first long vein, the basal one rather indistinct, the median spread across the wing-field as a faint dusky band; the third long vein is faintly darker than the rest. Wing fringe long and dense; first submarginal cell considerably longer and narrower than the second posterior cell, its base very slightly nearer the base of the wing than that of the second posterior cell; its stem about one-fourth the length of the cell, not quite so long as the stem of the second posterior cell; stem of the latter less than half of the cell; the second long vein carried a long way past the marginal cross-vein; supernumerary and mid cross-veins sloping towards the apex of the wing; posterior and mid cross-veins in one line. Halteres pale.

Length—2·5 mm. *Habitat*—Gambia (Dr. DUTTON). *Time of capture*—December.

Observations—Described from a single ♀ taken by Dr. DUTTON on the side of a tub on McCarthy Island. It is the only African *Corethra* known, and can easily be told by the wing ornamentation and

leg banding. The specimen is described partly from a xylol-balsam preparation. The mouth is provided with very distinct piercing lancets. It comes most near *Corethra brasiliensis*, but can at once be separated by the leg banding, wing venation, and spotting.

The great extension of the second long vein past the marginal transverse vein is a very marked character.

OTHER DIPTERA

Phlebotomus. Sp. ?

Several specimens of a large 'owl-midge' hatched out from pupae, taken in November and December from a duck pond. The species is probably new. It seems to be common in West Africa.

Tabanus dorsovitta. Walker

Two specimens of this common West African gadfly, which bites severely, taken in mangrove swamps and called by Dr. Dutton the large Mangrove fly.

Glossina longipalpis. Wiedemann
Var. *tachinoides.* Westwood

Eight of this Tsetse fly were taken by Dr. Dutton in November. It bites viciously along the river. It is closely related to the *Glossina longipalpis*, Wied., but constitutes a distinct variety. It was described by Westwood as a distinct species. Mr. Austen treats it as a variety of the type. Its possible connexion with the *Trypanosoma* in man has been referred to before.

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